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D P POLAROGRAPHIC ASSAY OF MINOXIDIL, ZOMEPIRAC SODIUM AND KETOPROFEN

by

(C) LAWRENCE AMANKWA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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Date....Nov. 4. 1983.....

Dedicated to my parents.

ABSTRACT

The polarographic behaviour of minoxidil, zomepirac sodium and ketoprofen has been studied and consequently, differential-pulse polarographic(d.p.) methods have been developed for the determination of these compounds in pharmaceutical dosage forms.

Minoxidil exhibits one well resolved d.p. polarographic wave in 1.0N H_2SO_4 with a E_p value at - 0.95V vs S.C.E. The d.p.p peak current varies linearly with the concentration of the drug over the range $1x10^{-5}$ to $5x10^{-4}$ M with a correlation coefficient of 0.9999. The electrochemical reduction of minoxidil has been found to involve the N-oxide bond as well as the 3,4-azomethine bond. In acidic solutions, the mechanism of reduction involves the transfer of four electrons as well as dehydration and deamination steps to yield 2-amino 6-piperidinopyrimidine as the final product. Cyclic voltammetry on minoxidil in 1.0N H_2SO_4 reveals that the compound is strongly adsorbed on mercury, whereas in an aprotic media a one electron quasi -reversible reduction process is predicted.

Zomepirac sodium and ketoprofen exhibit well developed d.c and d.p polarographic waves in Britton-Robinson buffer (containing 5% v/v methanol) over the pH range 6.0 to 11.0. Three waves were exhibited by each compound, however, the peak for quantitative estimation of zomepirac sodium was observed at pH 11.0 while, that for ketoprofen was observed at pH 6.0. The diffusion currents for the selected peaks vary linearly with the

concentration of these drugs with correlation coefficients of 0.9992 and 0.9994 for zomepirac sodium and ketoprofen respectively. The electrochemical reduction of these compounds involves the transfer of two electrons and the conversion of the benzoyl carbonyl group to the alcohol. In each supporting electrolyte system the reduction process has been found to be predominantly diffusion controlled.

The developed quantitative procedures are simple, rapid, accurate and they can determine the drugs to about 2mg/l levels. The procedures involve simple pulverization of the dosage form (tablet) followed by methanolic extraction of the active ingredient prior to the polarographic analysis in the appropriate supporting electrolyte system. Excellent agreements between results obtained by the developed methods and those provided by the manufacturer's control laboratories were realized. The methods show promising application for purity and stability studies and the commonly used tablet excipients and suppository bases do not interfere in this analysis.

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FUNDAMENTALS OF POLAROGRAPHY AND RELATED TECHNIQUES

1.

Since the inception of polorography by Prof. Heyrovsky (1), the technique has been through a series of modifications both in its principles and in its instrumentation. Direct current voltammetry at the dropping mercury electrode (D.M.E) or classical polarography involves the measurements of current voltage curves at the D.M.E. when a slow linear voltage ramp input (typically 100 mV/min) is applied to the cell.

The unique advantages of the D.M.E, (renewable, reproducible, and large negative potential range), are supplemented by the fact that well defined "S" shaped current voltage curves are obtained for most electrode process. The analytical parameters of interest being,

i) The current on the plateau of the wave, i_{lim}, which is linearly related to the concentration, C, by the Ilkovic equation (1) when the current is diffusion controlled:

$$i_d = 607.0\ n\ F\ C\ D^{1/2}\ m^{2/3} t^{1/6} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where

n is the number of electrons taking part in the electrode process.

F is the Faraday constant

D is the Diffusion Coefficient

m and t are the characteristics of the mercury flow rate and electrode drop time respectively.

ii) The half wave potential $E_{1/2}$, which is the potential where the current equals one half of the limiting value.

iii) The slope of the current potential curve.

The last two parameters can be used for identification of the molecule or species that is undergoing the electrochemical reactions.

Equation (1) is uncorrected for the value of the charging current. This charging curfent, of primary importance in polarography, is due to the adsorption of anions and cations at the electrode surface to form a double layer. This double layer has a finite capacitance and therefore a significant current is required to charge the electrode solution interface to the required potential. It is the existance of this charging current, which effectively limits the sensitivity of the d.c. method to about 5×10^{-5} M. This capacitive current is very large at the start of the drop life time but decreases with time during the drop growth. This important difference between the faradaic and capacitive currents is the basis on which most modern polarographic techniques have been developed.

The mercury flow rate m, and the dropping time t, are greatly influenced by the height of the mercury column h. The net pressure at the D.M.E. capillary tip is equal to the observed height of the mercury column minus the back pressure, which is due to excess pressure needed to overcome the interfacial tension of mercury and to expand the drop. The general equation can be summerized as follows:

$$h_c = h_o - 3.1/m^{1/3}t^{1/3} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

From the fact that an increase in the corrected height of the mercury column produces no increase in the mercury drop size, but rather causes an increase in the number of drops per unit time, relationships between mercury flow rate m, drop time t, and the corrected height of the mercury column can be expressed respectively as

$$m = K_1 h_c \quad \ldots\ldots (3)$$

$$t = K_2 / h_c \quad \ldots\ldots (4)$$

From equations 1, 3 and 4, a relationship between i_d and h_c could be derived as given by equation (5)

$$i_d = K_3 h_c^{1/2} \quad \ldots\ldots (5)$$

Equation (5) provides a means of ascertaining whether the current produced at the D.M.E. is actually the result of a diffusion controlled process. For a completely diffusion controlled process, a graph of i_d versus the corrected height of the mercury column is a straight line that passes through the origin.

Linear potential sweep chronoamperometry was one of the first of the modern variants to the classical polarographic method. The original method, often referred to as cathode-ray polarography was based on the application of a rapid voltage sweep to the electrode during the last part of the mercury drop life time. In linear sweep methods, peak response is obtained from the combined effects of high mass transfer rates in the non-steady state followed by the progressive depletion of the reactant concentration in the diffusion layer. Linear sweep techniques are not widely used

in modern polarographic analysis as the sensitivity is severely limited by the fact that the double layer charging current is also dependent on sweep rate as is the peak potential.

An important variant of linear sweep voltammetry is the technique of cyclic voltammetry, where the imposed signal consists of a triangular wave form with the first sweep being followed by a reverse sweep back to the initial potential. Important mechanistic information is obtained from the peak potential separation, the ratio of cathodic to anodic peak currents and the effects on these two parameters caused by variation in the voltage sweep rate.

All of the modern variants of the classical polarographic method have sought to improve sensitivity by eliminating the contribution from the capacitive current due to the double layer charging of the drop. While there is a vast array of techniques available, only a few have found routine acceptance. Pulse polarography as discovered by Barker *et al.*(2), involves the imposition of a series of increasing amplitude voltage pulses on successive drops. The current being sampled at the initial potential and after a selected time following the pulse application. Subsequently various modifications to the original technique have been made and, at the present time, pulse techniques are the most widely used in analytical polarography - voltammetry.

Utilising the differing time dependency of faradaic and charging currents, the pulse method imposes a series of

increasing amplitude voltage to successive drops at a preselected time near the end of each drop life time. The initially high charging current decays away very rapidly and the residual faradaic current is sampled during the final part of the 50-60msec time pulse. At a stationary electrode, the capacitive current i_c decays as an exponential function according to equation (6)

$$i_c = (\Delta E/R) \times e^{-(t/RxC_{dl})} \quad \ldots\ldots (6)$$

Where, ΔE is the pulse amplitude, R is the uncompensated resistance (dependent on cell and solution characteristics), t is the time elapsed since application of the pulse and C_{dl} the double layer capacitance.

By sampling the current for a short period - usually for a period equal to one cycle of the main supply, when the capacitive component has decayed to a steady state, a favourable measuremnt of the faradaic current over the capacitive current could be achieved.

Equation (7) compares the diffusion limited current for pulse and d.c. polarography.

$$i_p / i_{dc} = (3t_p / 7t_d) \quad \ldots\ldots (7)$$

Where t_p is the time after application of the pulse and t_d is the drop time in the d.c mode. While the ratio predicts that normal pulse will only be a factor of five to ten times more sensitive than d.c poloragraphy, in practice the ratio is nearly two orders of magnitude. The additional increase is due to the ability of the pulse technique to discriminate against the capacitive component.

Another advantage of the pulse technique is that as the measurement pulse is only applied for a small fraction of the total drop time, only a small amount of material is deposited on the electrode. Pulse polarography is extremely useful in analytical applications, since it can respond to both reversible and irreversible processes. While the normal pulse technique gives a marked improvement in sensitivity over the d.c method, it still gives a similar "S" shaped polarogram.

A much more useful variant, which currently is by far the most widely used polarographic technique for analysis, is differential pulse polarography. In this mode, a small amplitude (10-100mV) pulse of (ca 60msec) duration is superimposed on a conventional d.c. voltage ramp, and is applied to the D.M.E. near the end of the drop life time. The current output is sampled at two intervals, immediately on the ramp prior to the imposition of the pulse and again at the end of the pulse (after 40msec) when the capacitance current has decayed. The response signal is the difference in these two currents. The current-voltage curve, therefore, ideally has a "Gaussian-like" shape.

Parry *et al.*(3) have derived a relationship between the peak current i_p and the pulse modulation amplitude, ΔE. The maximum peak current when the pulse modulation amplitude is less than the value of RT/nF defined as :

$$i_p = n^3F^3A\,C \,/\, 4\,R\,T\,(D/\pi t)^{1/2} \times \Delta E \quad \ldots\ldots\ldots\ldots(8)$$

For very small values of ΔE the peak current potential coincides with the half wave potential, $E_{1/2}$. For larger values of the modulation amplitude, however, E_p is related to $E_{1/2}$ as given by equation (9)

$$E_p = E_{1/2} - \Delta E/2 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

It is therefore clear that maximum sensitivity in differential pulse polarography is obtained for large values of ΔE. Increases in ΔE, however, also result in increased peak broadening with consequent loss of resolution. For irreversible systems, however, peak current values are generally lower and peak widths broader than predicted for reversible values (3). Many analytical appplications of pulse and differential pulse polarography have been reported (4,5).

Other new improvements, on classical polarography are the alternating current techniques (6), stripping voltammetry (7,8) and coulometric techniques (9,10). For detailed discussions of these other techniques appropriate texts should be consulted (4).

1.1 Analytical Application

Voltammetric methods are found to be particularly rapid, convenient, accurate and precise in formulation analysis where frequently the reducible or oxidizable active ingredient can be determined in the presence of generally electroinactive excipients, irrespective of whether the latter are soluble or not in the chosen supporting

electrolyte. The procedures employed are simple and eliminate both the time consuming solvent extraction steps and calculation of recovery common to photometric and chromatographic methods, while the resulting accuracy and precision are at least comparable if not better than the aformentioned methods. Voltammetric methods can also be used as rapid means for stability testing of formulations provided that the degradation products are electroactive at different potentials or, alternatively electroinactive. *In-vitro* dissolution rate measurements can be followed easily by voltammetric sensors which do not require the need for serial sampling, extraction and tedious calculations often needed to perform dissolution rate determinations by photometric or chromatographic methods (4).

Direct voltammetric analysis of organic molecules in biological fluids is rapid and can be of great importance in *in-vivo* biological monitoring. These methods have been applied to some aspects of clinical diagnosis. When the molecule of interest has to be separated from the biological matrix then not only does the method become more time consuming, but the accuracy and precision of the overall method is adversely affected.

Voltammetric methods of analysis have sensitivities varying from 10^{-3}M (d.c) to 10^{-7} to 10^{-10}M for stripping voltammetry. Linear sweep and differential pulse polarography have sensitivities greater than spectrophotometry and equivalent to those of

spectrofluorimetry, G.L.C. with a flame ionization detector and liquid chromatography with a UV detector. Stripping methods can attain the sensitivity of either G.L.C. with electron capture or mass-spectrometric detector, or radioimmunoassay, all of which have sensitivities in the nanogram to picogram per ml range. The highly sensitive voltammetric methods have found application in pharmaceutical analysis for single tablet assay and in determination of small amounts of electroactive degradation products (4).

For trace determination of drugs and other organic molecules in biological fluids, highly sensitive procedures are required. Differential-pulse and fast linear sweep polarography are the voltammetric techniques normally used for the routine determination of drugs in blood or plasma. In addition, polarographic methods are ideally suited for the analysis of urinary metabolites since they are usually polar compounds. This is in contrast to G.L.C. methods which often require derivatization to reduce the polarity of the compounds to yield volatile derivatives suitable for analysis.

In general, voltammetric methods on their own are not particularly selective although some elegant examples to the contrary exist in the literature (11). Voltammetric methods can show exceptional specificity when the electroactive parent compound produces electroinactive metabolites or degradation products. Stripping methods have the ability to

discriminate between oxidation states of metals contained in some organometallic species, which is important in the study of their metabolic reaction in enviromental situations. In a recent investigation of d.p.p. as an identification tool in forensic drug analysis, it has been found in spite of the large number of drugs that are encountered in forensic science, that relatively few "acidic" and neutral drugs are reducible (12). Molecules which do not have inherent polarographic activity, or which exhibit behaviours that are of little analytical value can be subjected to a variety of complexation and derivatization procedures which can then be employed to achieve an indirect method of electrochemical analysis.

1.2 Quantitative Analysis

The magnitude of the diffusion currrent is related to the concentration of reducible or oxidizable species by the Ilkovic equation. There are, however, several ways of evaluating the unknown concentration of a substance by polarographic analysis. The most commonly used methods include:

(i) Absolute Method:

The absolute method is based on the direct application of the Ilkovic equation, however, because of the difficulty in evaluating the diffusion coefficient the method is not used to any extent in practical work.

(ii) Modified Absolute Method:

The Ilkovic equation can be rearranged to

$$KnD^{1/2} = i_d / m^{2/3} t^{1/6} C$$

Parameters which are independent of the electrode and instrument characteristics are grouped together on the left and are collectively referred to as the "Diffusion current Constant" I_d, which is frequently avialable in the literature with reproducibility within 5% under specified conditions. The method is not very accurate and it is also very time consuming.

(iii) Direct Comparison by Calibration Curve Method:

The concentration of an unknown solution can be determined by extrapolation from a previously prepared calibration graph. This method is convenient for the routine analysis of a large number of samples. Calibration curves should not be assumed to be valid form day to day, but should be verified for each series of analysis.

(iv) Alternate Direct Comparision Method:

This method is based on the simple relationship

$$i_d = KC$$

where

C = the concentration of the electroactive species

i_d = diffusion current

K = constant

for a standard solution and a test solution

$$\frac{(i_d)\ \text{sample}}{(i_d)\ \text{standard}} = \frac{C\ \text{sample}}{C\ \text{standard}}$$

Greatest accuracy is obtained when the concentration of standard and sample are approximately of equal value, especially for a slightly non-linear relationship between current and concentration.

(v) Standard addition method

In this method the diffusion current of the unknown solution with volume A is determined first and then a known volume B of standard solution is added to the unknown solution and finally the diffusion current of the mixture is measured. The unknown amount is then evaluated from the following equations

$$C_u = (C_s \times B \times i_u) / (i_m(A + B) - A \times i_u)$$

Where

C_u = concentration of the unknown test solution

C_s = concentration of standard solution

A = volume of unknown test solution

B = volume of standard solution

i_u = diffusion current of the unknown test solution

i_m = diffusion current of the mixed solution

Maximum accuracy is obtained with this method when the increase in wave height caused by the addition of the standard solution is approximately equal to that of the unknown test solution alone. The method is claimed to be more accurate than the calibration method, but more time consuming.

1.3 The Pilot - Ion Method

Since the diffusion coefficients vary from ion to ion, there is no uniformity in the heights of the waves obtained with equivalent concentrations of different reducible ions. The Ilkovic equation predicts constancy of the diffusion current constant for any given temperature. A knowledge of the diffusion current constant obviates the need for repeated calibration. This possibility cannot be fully realized since the values of m and t must be redetermined for each new capillary. However, the values for the various ions all change in the same proportion. Hence, once a series of these constants are determined for one capillary it is necessary only to repeat the determination of the constant for one ion in order to establish those of the entire series for a new capillary. This makes it necessary to maintain only a single standard stock solution for each supporting electrolyte likely to be needed. Relative diffusion current constant I_d of ions in a specific supporting electrolyte is independent of capillary characteristics.

To apply this method of quantitation, first determine the pilot ion ratio for the sample depolarizer and a chosen pilot ion. Secondly, determine the diffusion currents for the depolarizer and the pilot ion using a solution containing a known amount of the sample depolarizer. The concentration of the unknown sample depolarizer can be determined using the equation.

$$C_x = (i_x / i_p)(I_p / I_x) \times C_p$$

Where I_p/I_x is the pilot ion Ratio determined.

i_x is the diffusion current for the sample depolarizer

i_p is the diffusiin current for the pilot ion

C_x and C_p are the concentrations of the sample depolariser and pilot ion, respectively.

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MINOXIDIL

2.2 Introduction

Life expectancy is reduced in patients with elevated blood presure. Evidence has accumulated that reduction of elevated blood pressure reduces the risk of morbidity and mortality.

The drugs used in the treatment of hypertension belong to groups of distinct pharmacological actions, although the precise mode of action of some of them is not as yet fully understood.

Major drugs used in the treatment of hypertension are diuretic, and beta-adrenoceptor blocking agents. The most common agents can be broadly classified as:

i) adrenergic neurone blocking agents which interfere with postganglionic sympathetic nervous transmission but are without effect on the parasympathetic nervous system ; they include the guanidinium antihypertensive agents, bethanidine, guanethidine.

ii) Rauwolfia alkaloids and related compounds which have central and peripheral depressant action eg. deserpidin, reserpine, and rescinamine.

iii) Ganglion-blocking agents which interfere with nervous transmission of both sympathetic and parasympathetic ganglia. The effects are due largely to parasympathetic blockage eg. metaphan, mecamylamine.

iv) Alpha-adrenoceptor blocking agents eg. phenoxybenzamine, and prazosin.

v) Enzyme inhibitors such as methyldopa which probably act centrally.

Other new drugs which have been added to the list of antihypertensive agents include hydralazin, minoxidil and diazoxide, these appear to have predominantly peripheral effect.

Although, several potent antihypertensive agents are available, the blood pressure of some hypertensive patients cannot be controlled with standard drugs.

Minoxidil (Fig 1) (2,4-diamino-6-piperidinopyrimidine-3 -oxide) is an orally administered peripheral vasodilator that is useful in managing patients with refractory hypertension. Minoxidil lowers elevated systolic and diastolic blood pressure in the recumbent or standing positions and at rest or upon exertion. Its hypotensive effects result from vasodilation produced by a selective relaxation of peripheral arteriolar smooth muscle, thereby decreasing total peripheral vascular resistance (2,3). Lowering of blood pressure is accompanied by a variety of hemodynamic effects mediated by reflex sympathetic stimulation. Minoxidil causes sodium and water retention. In addition, minoxidil, like other vasodilators, elevates plasma renin activity (4,5). Subsequent production of angiotensin II stimulates aldosterone to retain sodium and water. These actions may lead to increased plasma volume, hemodilution and edema (6). The concomitant use of a sympathoplegic agent and a diuretic markedly reduces or

ameliorates these effects. One of the most embarassing side effects of minoxidil therapy is hirsutism or hypertrichosis, which occurs in about 80% of the patients taking the drug. Pericardial effusion has also been detected in some patients receiving minoxidil.

After oral administration, minoxidil appears in the plasma within 30 min, and maximum concentrations are achieved in about an hour. The average half life is 1.4 hr. for the parent compound and 4.2 hr. for the combination of parent drug and its metabolites (7). Sixteen hours after a single dose, the drug is not detectable in plasma. There is no accumulation of the parent drug after chronic dosing, however, glucuronide metabolites are retained in patients with impaired renal function. Minoxidil is eliminated in the urine mainly as three metabolites. The primary metabolite is a glucuronide conjugate that appears in the urine during the first 12 hrs. after administration. Ten percent of an administered dose of minoxidil is excreted unchanged in the urine. The other minor metabolites found in human biotransformation are the reduced forms of minoxidil, 2,4-diamino-6-(4'-carboxy,n-butylamino)pyrimidine, and 2,4-diamino-6-(4'-carboxy,n-butylamino)pyrimidine-3 oxide (8). In subacute toxicity studies after a one month oral dosage schedule, minoxidil was found to exhibit no evidence of gross or microscopic toxicity at a dose of 20mg/kg per day in four monkeys or four minipigs. At this dose level, a degenerative lesion confined to the right atrium of the

heart was noted in four dogs. Histologically, the lesion is charactarized by acute and chronic hemorrhage, degeneration of muscle cells and proliferation of blood vessels and connective tissues(9). Chronic toxicity studies of one year duration in three groups of four dogs each at oral dose level of 3, 10, and 30mg/kg per day of minoxidil also reveals right atrial lesions but no indication of other toxicity.(9) Because of these findings, the use of minoxidil in humans has been limited to patients with severe, refractory hypertension. The detection and determination of minoxidil continues to be of interest particularly because of its associated numerous side effects.

In the early stages of testing the toxicity and distribution of potential pharmaceutical compounds in experimental animals, the analytical method of choice usually involved a radiochemical technique. Bearing in mind current awareness of the dangers of the use of radioactive compounds and the need for rapid and complimentary "cold" methods of bio-assay other equally sensitive methods could be more extensively applied at this stage to appropriate compounds.

Accordingly, in this section, a differential-pulse polarographic procedure is presented for the determination of minoxidil in tablets.

Minoxidil occurs as a white or off-white odourless, crystalline solid that melts and decomposes at about 261°C. It has a molecular weight of 209.5 and is readily soluble in

water to the extent of approximately 2mg/ml, and in propylene glycol, ethanol and methanol. It is almost insoluble in acetone, chloroform, or ethylacetate. Minoxidil is prepared by a six step process starting with barium carbonate via guanidine hydrochloride and finishing with an overall yield of about 17 per cent (10) of the desired product.

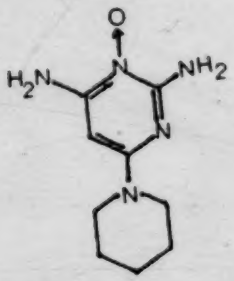


Figure 1

2.3 LITERATURE SURVEY

Numerous analytical techniques have been applied for the detection and determination of minoxidil especially in biological fluids. High pressure liquid chromatography (11), Thin-layer chromatography and radiochromatogram scanning (8,10), gas chromatography (11) and radioimmunoassay (11) are among the techniques so far reported in the literature.

Royer *et al* (11) have reported a radioimmunoassay technique for the determination of minoxidil in human serum. Antisera were developed in rabbits against two bovine serum albumin conjugates of the N-4-glutaryl and dipiperidine derivatives of minoxidil. These antisera were compared for specificity, and one was chosen for further development of the radioimmunoassay. ^{14}C-minoxidil derivatives were used. The technique offers a convenient means of determining minoxidil in human serum without administering radioactive minoxidil to the patient and without requiring subsequent chromatography of the metabolites. The technique has adequate specificity for measuring minoxidil in the presence of other metabolites, and no requirement for extraction or extensive sample preparation was necessary.It also has adequate precision and accuracy for comparing serum levels and determining serum half life, sufficient sensitivity and applicability to a large number of samples. The method however, is not suitable for analysis of pharmaceutical dosage forms.

In two other reports, Thomas *et al*.(10) and Thomas *et al*.(8) have applied radiochromatogram scanning and T.L.C. for the determination of minoxidil and its metabolites in test animals and in biological fluids respectively. Samples of urine or plasma containing sufficient radioactivity were applied directly to the paper or thin layer medium for chromatography. Paper chromatography was carried out on 86 x 15cm sheets by a descending technique in 1-butanol: piperidine: water (82:2:16v/v) system. Occasionally 1-butanol: acetic acid: water (4:1:1v/v) and benzene:methanol: ammonium hydroxide (100:100:1v/v) systems were used. The chromatograms were viewed under UV light. Ferric chloride spray reagent was also used to detect minoxidil and metabolites containing the N-oxide group. Attempts with G.L.C. procedures indicated that sizable amounts of the several derivatives investigated were lost on the column. H.P.L.C. would have adequate sensitivity for only the higher serum concentrations encountered at normal dosage levels of minoxidil.

None of the analytical methods so far reported has been directed towards the determination of minoxidil in pharmaceutical dosage forms.

Structural examinations of minoxidil reveals that the compound has two functional groups that can be utilized for analytical purposes. The N-oxide and the azomethine bonds in the molecules have been shown to be electroactive. Their electroactivities have been demonstrated by various workers

either qualitatively or quantitatively.

The polarography of pyrimidine and its derivatives at the dropping mercury electrode has been studied extensively by several workers (12-16). The most detailed study was that of Smith and Elving (14). This work included controlled potential electrolysis and coulometry as well as d.c polarography and resulted in the postulation of a mechanism for the electrochemical reduction of pyrimidine. Five polarographic waves have been observed for pyrimidine over the pH range 0.5 to 13.0. In highly acidic medium, a pH - dependent one electron wave I is observed. At about pH 3, a pH - independent one electron wave II emerges from the background discharge. These two waves merge near pH 5 to form a pH - dependent two electron wave III. Near pH 7.2, a pH independent two electron wave IV emerges from the background and at pH 9.2 merges with wave III to form the pH - dependent four electron wave V. Wave I is postulated to be the one electron reduction of pyrimidine to the neutral radical while wave II is the one electron reduction of the radical to a dihydropyrimidine. Wave III is the composite of these two steps. Wave IV is the two electron reduction of the dihydro - species to a tetrahydropyrimidine with wave V being the composite of waves III and IV.

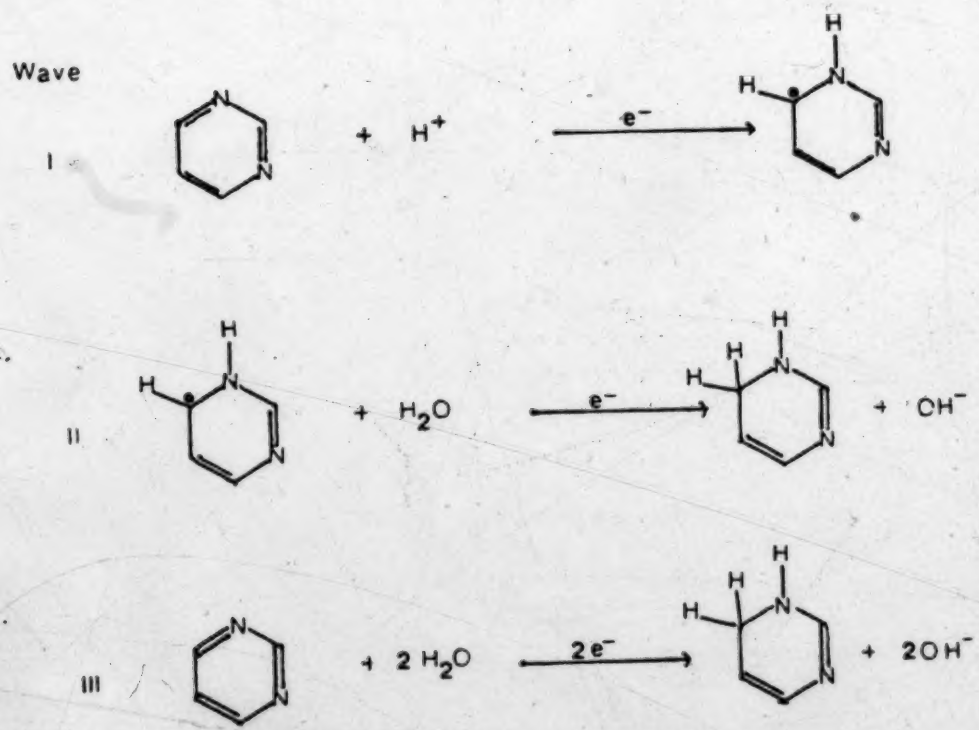
O'Reilly and Elving (12), in an effort to obtain further evidence for the proposed mechanism of pyrimidine reduction and to evaluate the various theoretical predictions for a rather complicated organic system,

subjected pyrimidine to a.c. polarography in aqueous media. Their findings generally substantiates the proposed reduction mechanism already obtained.Wave I is the most reversible of the pyrimidine waves, on the basis of proximity of $E_{1/2}$ and E_s values. The increased proximity of $E_{1/2}$ and E_s values with increased hydrogen ion concentration supports the postulate that proton addition is part of the overall first step, as more of the pyrimidine is protonated, the coupled chemical reaction of protonation becomes less critical in determining the rate of the first step. (pka for protonation of pyrimidine is 1.30). Wave II, associated with one electron addition to the neutral radical to form a dihydropyrimidine appears to be the most irreversible of the pyrimidine waves, as might be expected because of the stability of the dihydro species and the extreme difficulty of reoxidizing an aliphatic site. Wave II differs most from the other waves of the reduction path in that its first step involves attack on a neutral radical, whereas the initial step for waves I,II and V presumably involves initial attack on the 3,4-carbon-nitrogen double bond and that for wave IV on the 1,2-carbon-nitrogen double bond. The very similar characteristic of waves I and III are attributed to the fact that wave III involves the same initial step as wave I, ie., a one electron attack on the 3,4-carbon nitrogen double bond.

Waves IV and V, associated with the formation of a tetrahydropyrimidine species known to be unstable under the

alkaline conditions of its formation, are also highly irreversible.

Substitution effects on the polarographic behaviour of pyrimidine have also been investigated by many workers (14, 17-18) The ease of reduction of a pyrimidine generally decreases with the number of added amino and hydroxy substituents, the effect of which appears to involve saturation of the ring by means of tautomeric shifts, thereby removing possible reduction sites



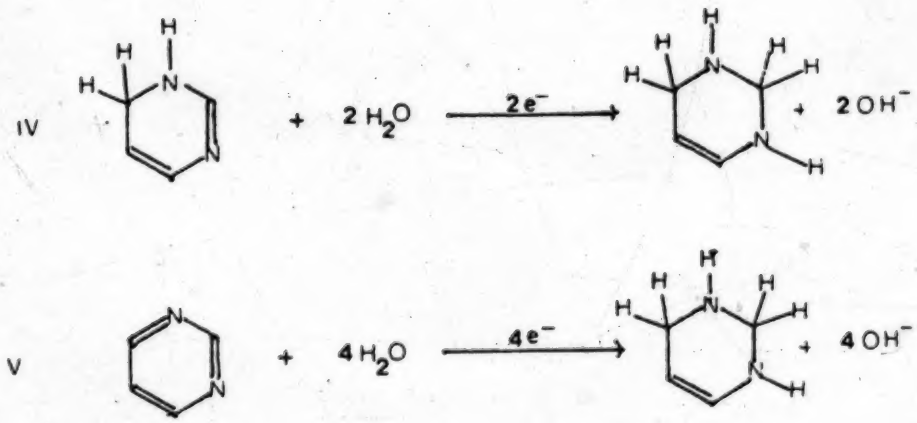


FIGURE 2: Proposed Reduction Mechanism of Pyrimidine Over the pH Range 0.5-12.0.

(Courtesy of O'Reilley and Elving (12))

None of the pyrimidines which are tri-and tetra-substituted with tautomeric groups are reducible. The

2,4-disubstituted compounds are either non-reducible or difficult to reduce. Pyrimidines having a 4-amino or 4-hydroxy substituent are more difficult to reduce than the corresponding 2-substituted compounds, but a hydroxy/ substituted compound is some what more difficult to reduce than an amino-substituted molecule. The greater stability of the 4-hydroxy substituted compounds has also been observed for the purine series (20). Hamer *et al*.(19), concluded on the basis of thymine and uracil being resistant to zinc reduction, that, the presence of oxygen at the 4-position confers some stability to the ring system. Ultraviolet absorption (21) indicates that all 2 and 4-hydroxy pyrimidines are largly in the keto form. The keto form of the 4-hydroxy compounds removes the N=C-C=C- system from the ring and replaces it with an O=C-C=C-system, where as if the equilibrium favours the imino form of the 4-amino compound, the former system is retained. Generally, pyrimidines substituted in the 4-position give higher currents than those substituted in the 2-position. 4-aminopyrimidines have been found to undergo deamination under polarographic conditions (14). The first step being the electroreduction of the 3,4-carbon nitrogen double bond to give 3,4-dihydro-4-aminopyrimidine. This intermediate is very unstable and undergoes rapid loss of ammonia to pyrimidine. The 2-amino susbtituted pyrimidines do not undergo the second 2e reduction which, for pyrimidine, involves the 1,2-double bond.

The polarographic behaviour of pyrimidine has been utilized for the determination of pyrimidine based biologically active compounds. Nucleosides and nucleotides have been determined in various sources, 6-azauridine mixtures (13), 6-azauridine in blood, purines and pyrimidine nucleosides and nucleotides in growing cultures of Escherichia Coli, and adenylic acid in hydrolysates of RNA in some tissues (22). Brooks *et al*.(23) have developed a differential-pulse polarographic procedure for the determination of trimethoprim in blood by utilizing the reducibility of the pyrimidine moiety. The supporting electrolyte was 0.1N H_2SO_4 and the $E_{1/2}$ for the analytical peak was - 1.07V *vs* S.C.E. The overall recovery of trimethoprim from blood and urine was 81.7% ±6.3 and 75.6 ± 7.2 respectively, while the sensitivity limit of detection from blood and urine is in the order of 0.5 to 0.75µg/ml. The correlation coefficient between a spectroflurometric and the d.p.p methods of assay is 0.90.

In another investigation on trimethoprim, Ellaithy and Volke (24) observed that well resolved waves were only formed in citrate HCl buffer pH 3.0-4.0. The reduction process was found to be irreversible and was initiated by the reduction of the 3,4-azomethine bond followed by the splitting off of the two amino groups. Coulometric experiments indicated that four electrons were consumed per molecule. Voltammetric oxidation of the drug at the rotating gold disc electrode yielded two analytical peaks. The exact

process for the oxidation is not fully understood since oxidation could occur at the pyrimidine ring or at the 3,4,5 trimethoxybenzene moiety of the molecule. Chatten *et al*.(25) recently reported a differential- pulse polarographic method for the determination of trimethoprim in pharmaceuticals. Sorenson acetate buffer pH 4.0 with 1M acetic acid was employed as supporting electrolyte. The resolution of the d.c. and d.p. polaraographic waves so obtained was much better than that previously reported by Ellaithy *et al*(24). Since acetic acid prevents the co-extraction of other tablet constituents which may interfere with the assay, a 1M solution of that acid was employed as the extracting solvent. Small amounts of 1% gelatin were also required to overcome the inconsistent suppresion of the d.p.p. peak height caused by the presence of povidone which co-extracts in acetic acid. Attempts to determine trimethoprim in the presence of sulphamethazole by anodic techniques proved to be difficult owing to the acid-base interaction between sulphamethazole and trimethoprim.

Smith (26) utilised the polarographic activity of the pyrimidine moiety to develop a method for the determination of the azidopyrimidine diuretic (2-amino-4-azido-5-(2-ethoxy ethyl)-6-phenylpyrimidine) in urine. The electrochemical reduction process was found to take place over a wide range of applied potential 200-300mV resulting in broad d.p.p. peaks with half widths in the order of 130-150mV. Improved resolution of the peaks could be obtained by increasing the

pH but this was associated with a decrease in the sensitivity of the procedure.

The second functional group in the minoxidil molecule which is also amenable to voltammetric analysis is the N-oxide bond. Voltammetric behaviour of N-oxide groups has been extensively studied by various workers (27-29). The N-oxide group has been found to undergo electrochemical reduction to give the free amine. Aromatic N-substituted oximes are reduced in acidic as well as basic media in a 4e wave to the secondary amine. The reduction proceeds through the corresponding Schiffs' base. Aliphatic N- substituted derivatives are reduced in acidic media only. Beckett *et al*.(29) analysed the polarographic wave of the N-oxide group and found the value of αn to be 0.50. Two steps were postulated for the reduction mechanism. The first was the rate determining step and involves a one electron and one proton mechanism. The second step is relatively rapid and it also involves an electron and a proton with the elimination of a molecule of water. The second step is so fast that it occurs at the same reduction potential as the first step, hence only one polarographic wave is observed. Beckett *et al*.(29) employed the reducibility of the N-oxide group in developing a method for the determination of the N-oxide, N-oxide sulphoxide and sulphoxide metabolites of chlorpromazine. The method can determine the N-oxide metabolite in the presence of the parent compound and the sulphoxide of chlorpromazine. This determination is

unsuitable with UV since the chlorpromazine N-oxide exhibits maxima at the same wavelength as the sulphoxide of chloropromazine. Gas liquid chromatography of chlorpromazine-N oxide produces an elimination product, 2-Cl-10-allyl phenothiazine in addition to some reduction products (30). Broad peaks and relatively long retention times preclude the use of G.L.C. for the determination of low levels of the chlorpromazine sulphoxide metabolites.

Brooks *et al*.(31), have reported a d.p.p. method for the quantitative determination of trimethoprim and its N-oxide metabolites in the urine of man, dog, and rat. The $E_{1/2}$ value for the N-oxide is well removed from that of the azomethine reduction. Phosphate buffer, pH 3.0, was used instead of 0.1N H_2SO_4. This enhanced the separation between the $E_{1/2}$ values for the N_1- oxide and the N_3-oxide. The method is sensitive and can be applied to follow the biotransformation and metabolic pathway of various N-oxide based biologically active compounds.

Several other workers (32-34) have examined the polarographic behaviour of chlordiazepoxide in a variety of supporting electrolytes. In acid solutions, three well defined polarographic reduction waves attributed to the reduction of the N-oxide, the 4,5-azomethine and the 1,2-azomethine bonds were observed.

Caile *et al*.(35) have assayed chlordiazepoxide in dosage forms by spectrofluorometric and polarographic methods and found that both methods yield comparable

results. Jacobson *et al.*(30) also reported an assay method by polarography for chlordiazepoxide in dosage forms. The method has a distinct advantage over the UV method in that the extraction of the drug into an organic solvent and removal of insoluble material was not required. Chlordiazepoxide tablets containing propantheline bromide can be assayed by this method since propantheline shows no polarographic activity although propantheline and chlordiazepoxide show similar UV absorbance.

2.4 Statement of Purpose

The few analytical methods reported for the determination of minoxidil involve a T.L.C, Radioimmunoassay, and Radio chromatogram scanning. These were mainly applied in biotransformation studies of minoxidil. Application of G.L.C. and H.P.L.C. techniques have been found not suitable for minoxidil determination. No official method has so far been listed for the determination of minoxidil in dosage forms. This present work is directed toward the investigation of the polarographic behaviour of minoxidil, and to the development of a differential-pulse polargraphic technique for the determination of minoxidil in dosage forms.

In order to accomplish this, the variables to be investigated include:

i) The determination of the optimum buffer or supporting electrolyte system; that which offers a strong

and well resolved polarographic wave for minoxidil.

ii) The effect of pH on the $E_{1/2}$ of the wave.

iii) The determination of diffusion dependency by plotting the diffusion current vs square root of the corrected height of mercury column.

iv) To investigate the electrochemical behaviour of minoxidil by cyclic voltammetry, and to determine the effect of scan rate on the cyclic voltammetric peak height.

v) To determine the number of electrons transferred per molecule during the electroreduction process.

vi) To perform a macroscale electrolysis of minoxidil, and to isolate and characterize the reduction product by means of mass spectrometry, N.M.R., IR., UV. and simple chemical reactions.

vii) To propose a mechanism for the electrochemical reaction.

viii) To prepare a calibration graph and to determine the coefficient of correlation beteween peak current and concentration of minoxidil.

ix) To apply the developed method to the analysis of pharmaceutical dosage form.

EXPERIMENTAL

2.5 Apparatus and Conditions for Polarographic Analysis

Standard laboratory glass ware, one meter rule, stop watch, nitrogen tank, Mettler balance, magnetic stirring device. Unicam SP1800 UV spectrophotometer and recorder. Perkin Elmer 267 IR spectrophotometer, Unicam SP1000 IR spectrophotometer, 200MHz and 60MHz NMR spectrometers. Other apparatus will be discussed under appropriate sections.

Reference Standard

Minoxidil (100.4%) was obtained from Upjohn Company, Canada Ltd., and used without further purification.

Reagents

The following reagents were used, all of analytical-reagent grade : barbital, boric acid, citric acid, potassium hydrogen phosphate, dimethylformamide, anhydrous methanol, tetraethylammonium bromide, 0.2N sodium hydroxide, 1.0N sulphuric acid, and 1% tetraethylammonium bromide in DMF. Britton-Robinson buffers were prepared with distilled, deionized water at intervals of 0.5 pH unit over the pH range of 2.6 - 7.0.

Pharmaceutical Dosage Forms

Name	Manufacturer	Dosage Form	Lot No
Minoxidil (Loniten)	The Upjohn Company of Canada	2.5 mg tablets	H - 651
Minoxidil (Loniten)	The Upjohn Company of Canada	10 mg tablets	H - 710

2.6 PROCEDURE

2.6.1 A. Determinatioin of Reference Standard Purity :

The purity of the reference standard minoxidil (100.4%) was checked by means of thin layer chromatography. Microsilica gel plates were used while the solvent system consisted of methanol: ammonia (100:1.5). Only one spot with an R_f value of 0.43 was observed. The reference standard was, therefore, used in subsequent experiments without further purification.

2.6.2 B. Constant Temperature Control for Polarography

The polarographic cell was immersed in a water bath connected to a thermostatically controlled reservoir. Circulation was ensured by means of a pump. The temperature of the water was maintained at a constant value of 25 ± 1°C.

2.6.3 C. Determination of Optimum Height of Mercury Column

Minoxidol solution (5×10^{-4} M) was prepared by diluting nineteen ml of 1.0N H_2SO_4 with 1ml, of 10^{-3} M methanolic solution of minoxidil in the polarographic cell. After deaeration for 10 min with purified nitrogen, the polarograms were run separately at various heights of the mercury column ranging from 60cm to 80cm with 5cm as the interval. The dropping time of the D.M.E. was maintained at 2s by an automatic drop timer. Comparison of the polarograms was made and the optimum height of mercury column was selected.

2.6.4 D. Determination of Optimum Buffer or Supporting Electrolyte System

Four systems were investigated in order to choose the one that is optimum for the analysis. Those investigated include, Walpole's acetate buffer pH 3.6 - 5.6, Sorenson's citrate buffer pH range, 1.2 - 5.6, Britton-Robinson buffers pH range 2.6 - 7.0 and 1.0N sulphuric acid, pH ≃ 0.5.

Minoxidil solutions (5×10^{-4} M) were each prepared by adding 1ml of 10^{-3} M methanolic solution of minoxidil to

19ml of the appropriate system in the polarographic cell. Polarograms were obtained in each instance after deaeration for 10 min with nitrogen. For the buffer systems, the pH was varied over the corresponding range in intervals of 0.5 pH units. The quality of the polarograms was compared to select the optimum system for the analysis.

2.6.5 E. Determination of Solution Stability :

The stability of stock solution of minoxidil was determined by running the polarogram on a freshly prepared solution and then allowing the solution to stand for five days before obtaining the second polarogram. A comparison of the two polarograms was made.

2.6.6 F. Determination of pH Change Before and After Electrolysis :

The pH values of the solution were measured electrometrically prior to and after the polarographic reduction, in order to ensure that the pH had remained relatively constant.

2.6.7 G. Comparison of Polarograms of Pyrimidine and Derivatives

The polarograms of pyrimidine and some selected derivatives were obtained in two supporting electrolyte systems, 1.0N H_2SO_4 and Britton-Robinson buffer pH range 2.6 to 11.0. The derivatives investigated include :

2,4-diamino-6-hydroxypyrimidine

4-amino-2,6-dimethylpyrimidine

2-amino-4,6-dimethylpyrimidine

4-methylpyrimidine

2-aminopyrimidine

In each instance, 1 ml of 10^{-3} M methanolic solution of the selected substance was added to 19ml of the appropriate buffer or other supporting electrolyte to produce a final concentration of 5×10^{-4} M.

2.7 Differential Pulse Polarographic Analysis

A Fisher, Model 320, pH meter fitted with a glass - calomel electrode system was employed to measure the pH values of the solutions.

A PAR, Model 174, polarographic analyser equipped with a drop timer (Model 172A) and a Houston Ominigraphic, Model 2000, recorder were used in the investigations. A three-electrode combination was employed which consisted of a saturated calomel electrode, a dropping mercury electrode and a platinum wire as the auxiliary electrode. A conventional H-type cell was maintained at 25 ±1°C and all sweeps utilized a scan rate of 2mV/s and a drop time of 2s.

In 1.0N H_2SO_4 (pH 0.5) the instrumental parameters were : applied potential range -0.6 to -2.1V; current 100μA full scale; height of mercury column 75cm; flow rate of mercury 1.176mg/s ; modulation amplitude, set at 50 mV, and low pass filter, set at a time constant of 1s. The instrument was

operated in the differential-pulse mode.

2.7.1 Controlled - Potential Coulometry

A PAR, Model 173, potentiostat-galvanostat, equipped with a PAR, Model 377A, three component coulometric cell system was connected to a Hi-Tek digital integrator and digital voltmeter.

Nineteen ml of 1.0N sulphuric acid were placed in the coulometric cell on top of a 5ml layer of triple distilled mercury and 1ml of 10^{-3} M solution of minoxidil in methanol was added. The system was purged for 10 min with purified nitrogen. The applied potential was set at -1.2V with a current range of 10μA full scale and the solution was electrolysed until the digital readout indicated a constant but small count. One hour was required to complete the electrolysis. The process was repeated with a blank consisting of 19ml of 1.0N sulphuric acid and 1ml of methanol. Experimental data obtained are presented in Table 3.

2.7.2 Cyclic Voltammetry

Cyclic voltammetric experiments at a hanging mercury drop electrode were performed with a four-component system consisting of a PAR EG and G, Model 175, Universal Programmer, a PAR, Model 173, potentiostat-galvanostat, a Houston, Model 2000, Omnigraphic recorder and a PAR, Model 9323, hanging mercury drop electrode fitted with a

polarographic cell. Two supporting electrolyte systems were employed. In 1.0N sulphuric acid, the instrumental parameters were : potential range -0.8V to -1.3V; current range, 10μA; scan rate varied from 10mV/s to 200mV/s . In a dimethylformamide/tetraethylammonium bromide system, the settings were : potential range, -1.2 to -2.2V; current range, 10μA; scan rates were the same as in the previous system.

2.7.3 Preparation of Calibration Graph

A stock solution of minoxidil (10^{-3} M) was prepared in anhydrous methanol. Five test solutions of varying concentrations, 1 to 5 x 10^{-3} M were prepared by appropriately diluting the stock solution with 1.0N sulphuric acid, while in the total sample volume of exactly 20ml, the amount of methanol was always maintained at 1ml.

All samples were purged with oxygen-free nitrogen for 10 min prior to each run and a stream of nitrogen was allowed to flow gently over the surface of the solution during the electroreduction. Samples of each of five concentrations were run five times and resulted in a correlation coefficient for the graph of 0.9999. Data and the resultant calibration graph are presented in Table 2 and Fig. 4 respectively.

2.7.4 Diffusion Dependence Studies

These studies were carried out in the aforementioned sulphuric acid-methanol system on a 5 x 10^{-4} M solution of minoxidil. The applied potential was from -0.6 to -2.1V and the height of the mercury column ranged from 60 to 80cm. The mercury flow rate was measured at each of five heights over that range.

2.7.5 Analysis of Pharmaceutical Dosage Forms

Two dosage forms, 2.5 and 10.0mg tablets, were available from the manufacturer.

Twenty tablets were weighed, finely powdered and an amount of powder was taken which according to the label would result in approximately 10^{-3} M solution of minoxidil in 50ml of solvent. The accurately weighed sample was stirred magnetically for 20 min in 20ml of methanol. The mixture was quantitatively transferred into a 50ml volumetric flask, diluted to volume with methanol and then filtered through a Whatman No.1 paper discarding the first 5ml of the filtrate. A 0.6ml aliquot of the filtrate was transferred to the polarographic cell, 19ml of 1.0N sulphuric acid and 0.4ml of methanol were added. As previously described, the solution was purged for 10 min with purified nitrogen prior to recording the polarogram.

The amount of minoxidil was computed from a calibration graph. (see Table 6).

2.7.6 Content Uniformity Test

Ten tablets were randomly selected from the sample. Each tablet was placed in an individual 150ml beaker, 20ml of methanol were added and the system was allowed to stand for 5 min in order to promote disintegration of the tablets. The remaining larger lumps of tablet mass were crushed with a glass rod and the mixture stirred magnetically for 20 min. After transferring the mixture quantitatively to a 50ml volumetric flask, the determination was continued as described in the previous section, except that 1ml of the filtrate and 19ml of 1.0N H_2SO_4 were used. The amount of minoxidil in each tablet was computed by the direct comparison method, using reference standard solutions of 0.2386×10^{-3} M and 0.9546×10^{-3} M for the 2.5mg and 10mg tablets, respectively .(see tables 4 and 5)

2.7.7 Macroscale Electrochemical Synthesis Of 2-amino-6-piperidinopyrimidine

The procedure for the macroscale eletrochemical synthesis of 2-amino-6-piperidinopyrimidine from minoxidil was similar to that for the controlled potential coulometry with the exception that the cell contained 150mg of minoxidil in 25ml of 20% v/v methanol in 1.0N H_2SO_4.. The applied potential was held at -1.2V and the reduction time was 6hr. Upon completion of the reduction, the product, together with the supporting electrolyte, was separated from the mercury, the pH adjusted to 7.0 with ammonium hydroxide

and the resulting solution extracted with chloroform. The organic layer was separated, dried over magnesium sulphate and concentrated to 1ml. The concentrated solution was applied to a 1mm thin-layer silica gel plate and then developed with a methanol: ammonium hydroxide solvent system (100 : 1.5). Two spots were observed. The R_f value of one corresponded to that of minoxidil while the other component with an R_f value of 0.60 was scraped off the plate, leached out with methanol and the methanolic solution was evaporated to dryness.

This isolation yielded a yellow, crystalline powder which decomposed between 80-100°C. NMR (200MHz, $CDCl_3$); δ 1.6 (m,6H), 3.5 (m,4H), 5.9 (m,3H) and 7.9 (d,1H)(see discussion); M^+m/e 178; IR (KBrdisc): 930/cm , 1090/cm , 1400/cm ,1680/cm , and 3000/cm The IR, NMR and Mass spectra of minoxidil and those for 2-amino -6-piperidinopyrimidine are presented in Figures 15 to 21.

Results and Discussion

2.8

2.8.1 Temperature Control for Polarographic Experiments

Normally, temperature should have little effect on the electron transfer of an electro-organic reaction, however, electrode potentials do shift with changes in temperature. The major effect of temperature is on the course of a follow up chemical reaction and on the stability of intermediates and product. The solubility of various species in the medium will, of course, also be a function of temperature. The conductivity of most electrolytes increases with increasing temperature by 1-2% per degree rise in temperature. For a reproducible polarographic wave, therefore, a temperature control system which can maintain the temperature of the cell at a constant value throughout the experiment is necessary. In this experiment the cell was maintained at 25 ± 1°C which resulted in consistent and well developed polarographic waves.

2.8.2 Determination of Optimum Height of Mercury Column

No significant differences were observed between polarograms procured at various heights of the mercury column. The height was varied over the range from 60cm to 80cm with a 5cm interval between settings. Well developed d.p and d.c. polarographic peaks were exhibited in each instance. Since an automatic drop timer was used to control the dropping time, the effect of altering the height of the

mercury column resulted only in small changes in the peak heights. A mercury column with a height of 75cm was chosen because it yielded well-resolved, easily-measured peaks and resulted in a rate of 1.176mg/sec at a droptime of 2s.

2.8.3 Determination of Optimum Supporting Electrolyte System

Each of the buffers investigated as well as 1.0N H_2SO_4 were screened polarographically and were found not to contain any substance that might interfer with the reduction of minoxidil. In both Walpole's acetate buffer pH 3.6-5.6, and Sorenson's acetate buffer pH 1.2-5.6, minoxidil does not exhibit any polarographic peak which is of analytical value. The waves do not have a limiting current plateau, and they are also poorly resolved from the back ground discharge current. Polarograms obtained with Britton-Robinson buffer pH range 2.6-7.0 show an improvement in the shape of the waves, especially at a pH of 5.0 (Fig 13, tracing C). This peak however, is not sufficiently well-resolved to be utilised for analytical purposes. The inability of minoxidil to exhibit well - defined polarographic waves in these buffers might suggest that the buffers have the ability to exert a profound effect upon the electrochemical behaviour of minoxidil.

In the 1.0N H_2SO_4 system, however , well-defined d.c. and d.p. polarographic waves were observed for minoxidil. The waves have an extended limiting current plateau and are also very intense (Fig 13, tracing A). The ability of

minoxidil to undergo reduction easily in highly acidic medium indicates that the protonated form of minoxidil is more amenable to reduction than is the unprotonated minoxidil. Therefore, the supporting electrolyte chosen for the analysis was 1.0N H_2SO_4.

2.8.4 Determination of Solution Stability

A comparison of polarograms obtained using a freshly prepared solution of minoxidil and that of a five day old solution showed no significant differences. Therefore, minoxidil is stable in methanol for at least five days. Consequently frequent preparation of stock minoxidil solution is not necessary.

2.8.5 Determination of pH Change Before and After Electrolysis

The pH of the buffered minoxidil solution does not show any significant change upon completing the polarogram. Small changes of about 0.5pH units were observed in some instances, however, these were not large enough to alter the shape of the polarographic wave or its $E_{1/2}$ value. The pH of each buffered solution prior to running the polarogram was, therefore, recorded as the pH at which the electrochemical reduction took place in that system.

2.8.6 Determination of Diffusion Dependency

The nature of a polarographic wave depends on the process governing the value of the limiting current. The most common types of polarographic limiting currents observed in the presence of electroactive species are diffusion, kinetic, catalytic and absorption currents. These can be distinguished by following the effect of changes in the concentration of the electroactive species under study, and the height of the mercury column.

Additional information may be obtained by observing how wave height are affected by altering the pH, temperature and composition of the system. An increase in the concentration of the electroactive species causes diffusion currents and most kinetic currents to increase linearly with the increasing concentration. Catalytic currents usually reach a certain limiting value as the concentration is increased, while adsorption currents are practically independent of concentration changes.

Generally, increasing the height of the mercury column results in an increase in the limiting current. Diffusion currents are a linear function of the square root of the corrected height of the mercury column. The height of the mercury column must be corrected for the back pressure of the solution. In addition, the extrapolated plot of the diffusion current against the square root of the corrected height of mercury column must pass through the origin in order to categorize a process as completely diffusion

controlled. A limiting current that is governed solely by the chemical reaction rate (ie a pure kinetic current) does not change with the change of mercury pressure.

In most instances, a completely diffusion controlled process or a completely kinetic controlled process is not obtained, hence plots of diffusion current versus the corrected height of the mercury column have slopes less than one but greater than zero and do not pass through the origin. For catalytic currents, various types of relationships between the limiting current and the corrected height of mercury column can be obtained, depending on the compound involved and on the conditions used. Adsorption currents sometimes give linear plots of current against corrected height of mercury column.

Temperature and pH effects on the limiting current can also lead to important information about the nature of the limiting current.

Adsorption and diffusion currents are rarely pH dependent and are almost always independent of the composition of the system, while kinetic and catalytic currents are often a function of pH. Diffusion currents are increased by about 2% per degree rise in temperature. Kinetic currents show greater increases with changes in the temperature, hence a current temperature coefficient which is large, in all probability, is a kinetic current. Some adsorption currents, however, decrease with increasing temperature.

In order to investigate the nature of the limiting current observed for the reduction of minoxidil in 1.0N H_2SO_4, a plot of the diffusion current versus the square root of the corrected height of mercury column was prepared (Figure 3). Data for obtaining the above graph are presented in Table 1.

The plot is a straight line with a slope of $2.02\mu Acm^{-1/2}$ but does not pass through the origin. From the foregoing discussion, it is apparent that, while the reduction process is largely dependent upon diffusion, some other parameter or parameters are also influencing the mechanism. The reduction might not be strictly electrochemical and some intermediate chemical reactions are predicted. The mercury flow rate varied with the applied potential and consequently the potential was maintained at the $E_{1/2}$ value of the polarographic wave for all measurements.

2.8.7 Preparation of Calibration Graph

A linear and reproducible calibration graph was obtained using pure reference standard minoxidil supplied by the manufacturer. A coefficient of correlation of 0.9999 was obtained between the d.p.p. peak and the concentration of minoxidil in the concentration range investigated ($1x10^{-4}$ to $5x10^{-4}$). Data for the preparation of the calibration graph and the resultant graph are presented in Table 2 and Figure 4 respectively. Extrapolation of the graph results in a line which does not pass through the origin. This observation

Table : 1

Data for Determination of Diffusion Dependency for Minoxidil.

Observed Hg Height (cm)	m in mg/sec	t in sec	Back pressure $h = 3.1/m^{\frac{2}{3}} t^{\frac{1}{3}}$	Corrected Hg Height	$\sqrt{}$Corrected Hg height	Diffusion Current(μA)	Average in (μA)
80.0	1.402	2.0	2.196	77.80	8.82	6.73 6.60 6.50	6.64
75.0	1.176	2.0	2.329	72.67	8.52	6.11 5.95 5.82	5.98
70.0	1.140	2.0	2.354	67.65	8.22	5.36 5.29 5.30	5.32
65.0	1.020	2.0	2.445	62.56	7.91	4.80 4.75 4.73	4.76
60.0	0.916	2.0	2.578	57.42	7.58	4.38 4.38 4.38	4.38

Table : 2

Data for Calibration Graph for Minoxidil

Conc of Drug x 10^{-5} M	Total Current Measured at -0.95V vs SCE(μA) 1	2	3	4	5	Average (μA)	i_p
1.0	1.90	1.90	1.90	1.90	1.85	1.89	
2.0	3.60	3.70	3.60	3.60	3.70	3.64	
3.0	5.20	5.20	5.30	5.40	5.40	5.30	
4.0	6.70	7.10	7.00	6.90	6.90	6.92	
5.0	8.80	8.70	8.40	8.40	8.74	8.60	

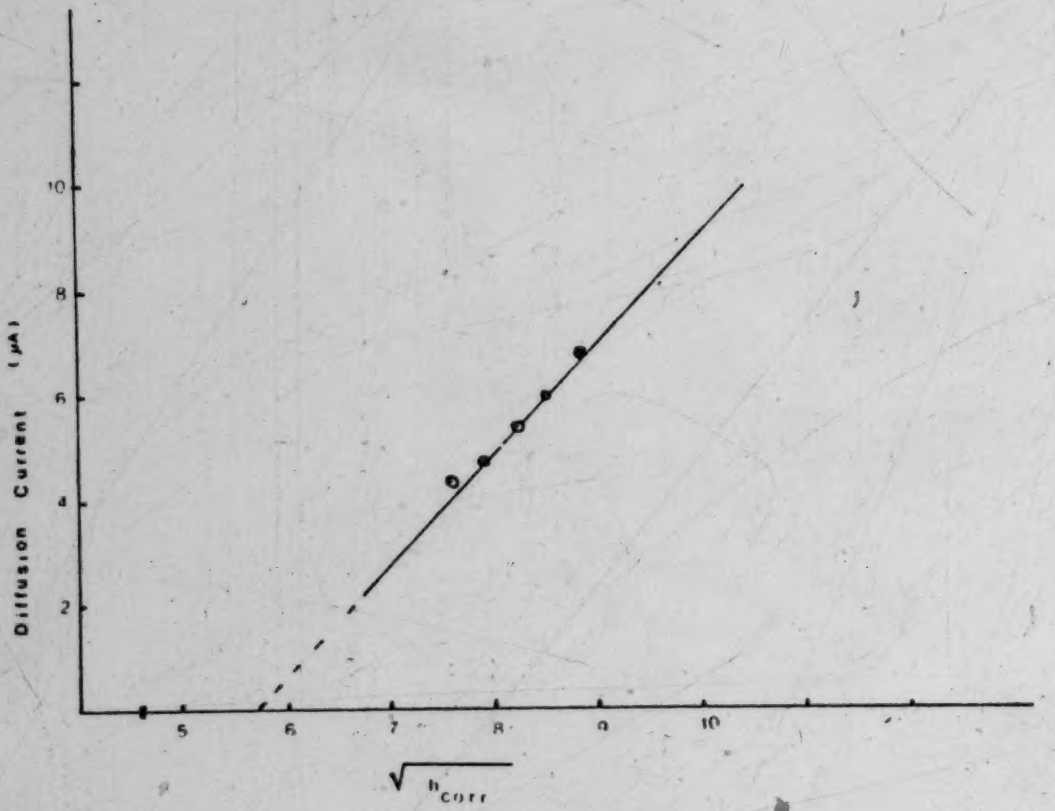


FIGURE 3: Graph of Limiting Current of Minoxidil (5×10^{-4} M) in 1.0N H_2SO_4 vs the Square Root of Corrected Height of Mercury Column.

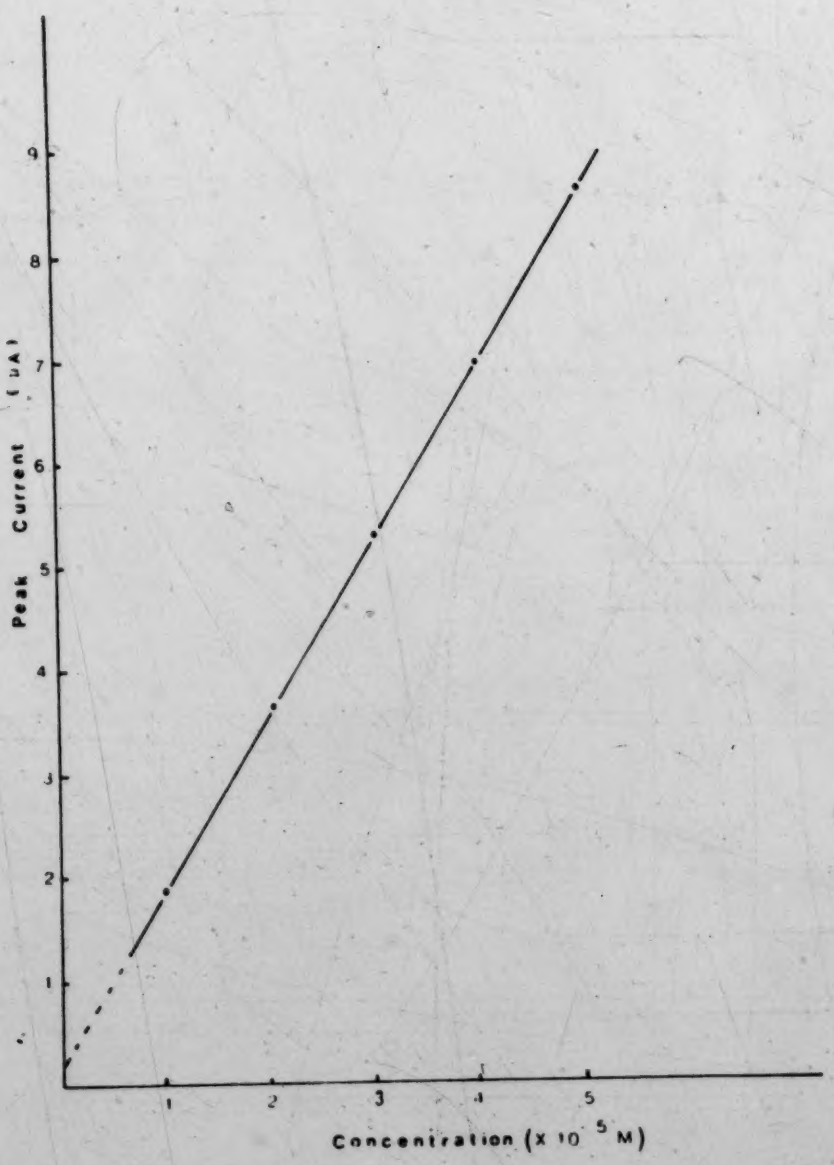


FIGURE 4: Calibration Graph for Minoxidil

suggests that the electrochemical process taking place at the D.M.E. is essentially, but not completely, diffusion controlled. Minoxidil can, therefore, be quantitatively determined by a d.p.p. technique in view of the high correlation between peak current and concentration of minoxidil.

2.8.8 Controlled Potential Coulometry

In their own right, coulometric techniques play an important role as analytical procedures and their use in providing supporting mechanistic evidence for polarographic and voltammetric studies cover only a small fraction of their total range of application.

Coulometric methods for mechanistic studies fall into two groups. Firstly, there are the absolute methods where the coulometric measurement is carried out at the microelectrode of interest, for example, at the D.M.E. (microcoulometry). In the second group of macro coulometric procedures, the measurement is carried out at a large area electrode, such as the mercury pool (macrocoulometry) and the evidence in terms of the number of electrons and identification of products is used as additional supporting evidence for the proposed mechanism of the electrode process of interest. It should be pointed out, however, that in the latter approach the mechanisms of complex reactions at a large area electrode are often quite different from analogous results obtained at a microelectrode owing to the

differing concentrations of reactive intermediates that would occur at the electrode surface in each instance.

In controlled potential coulometry the potential of the working electrode is controlled at a suitable potential on the plateau of the limiting current of the sample species of interest. The cell current decays exponentially with time to a constant back ground level.

The controlled potential coulometry of minoxidil (5 x 10^{-4} M) revealed that four electrons per molecule were involved in the electroreduction process. The reduction of minoxidil in acidic solutions at the D.M.E. probably involves two bonds, since each bond contains 2 electrons. From the structure of minoxidil, those most likely to be reduced are the N-oxide bond and the 3,4-azomethine bond. The data for the calculation of the number of electrons involved in the process is presented in Table 3.

2.8.9 Cyclic Voltammetry

Cyclic voltammetry is an important variant of linear sweep voltammetry whereby the potential is changed linearly with time. The change could be unidirectional, or the potential can be cycled between anodic and cathodic limiting values. Anodic and cathodic peaks are produced by the combined effects of high mass transfer rates in the non-steady state followed by the progressive depletion of the reactant concentration in the diffusion layer. Although quantitative data often can be obtained from cyclic

Table 3

Coulometric Data for Determining the Number of Electrons Transferred in the Electro-Reduction Process

Compound	Volume of solution V (L)	Volt sec for Blank Soln $\times 10^1$	Average volt sec for Blank A $\times 10^1$	Sample conc C (M)	Volt sec for Sample $\times 10^1$	Average volt sec forsample B $\times 10^1$	A - B $\times 10$	Current range (A)	Coulombs Q	Faraday F	Number of electrons
Minoxidil	0.02	2.250	2 260	5×10^{-4}	6 488	6.532	4.271	0.1	4.272	96487	4.4
		2.263			6.500						
		2.267			6.608						
2,4 - diamino 6 - hydr oxy pyri midine	0.02	78.080	70.08	5×10^{-4}	90.820	91.13	21.08	0.01	2.105	96487	2.20
		72.200			93.760						
		60.04			88.820						

voltammetry, one of its major uses is in the rapid qualitative elucidation of reaction mechanisms.

The behaviour of a system can be seen over a wide range on a single voltammogram; intermediates can be directly observed and possibly identified by the potentials at which they oxidise or reduce. The participation of preceding or following chemical reactions can be seen by altering the sweep speed to compete with the rate of the chemical reaction and by varying the potential sweep range.

General rules for interpreting cyclic voltamograms can be formulated as follows:

i) A simple electron transfer step for which the reactant supply is limited only by the diffusion rate always gives ($I_p / \nu^{1/2}$) independent of ν (scan rate). Deviations from this relationship implies coupled chemical steps (or adsorption). In particular, I_p actually decreases with increasing ν or increases less rapidly than $\nu^{1/2}$ when the reactant is supplied by a preceding chemical reaction. If I_p increases more rapidly than $\nu^{1/2}$, a following chemical reaction is implicated.

ii) The variation of E_p with while $I_p / \nu^{1/2}$ remains almost independent of ν is caused by a slow electron transferstep. Coupled chemical rections can also produce changes in E_p with ν but $I_p / \nu^{1/2}$ will not be independent of ν and the ratio of I_{p_c} / I_{p_a} will vary with ν.

iii) For peaks caused by adsorption steps, I_p is directly proportional to ν and the number of coulombs

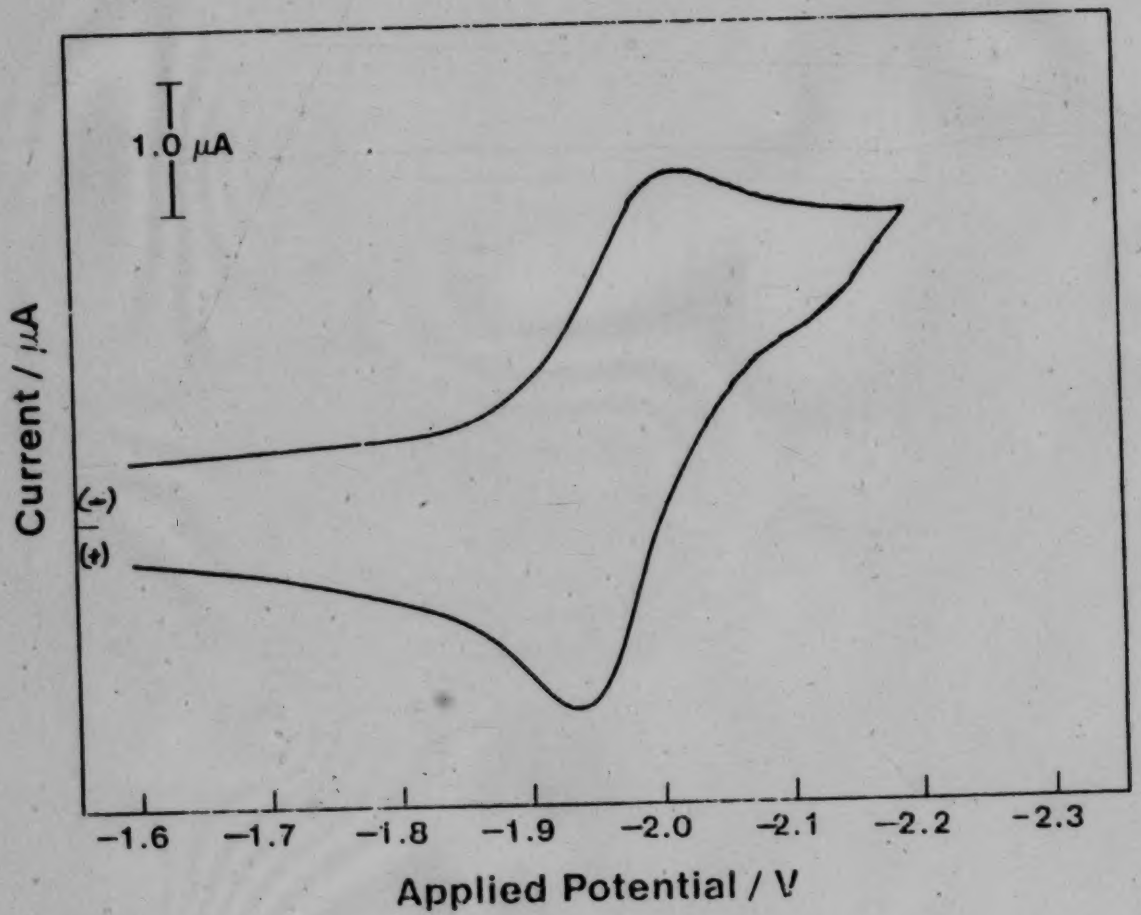


FIGURE 5: Cyclic Voltammogram of Minoxidil (5 x 10^{-4} M) in DMF/TEAB

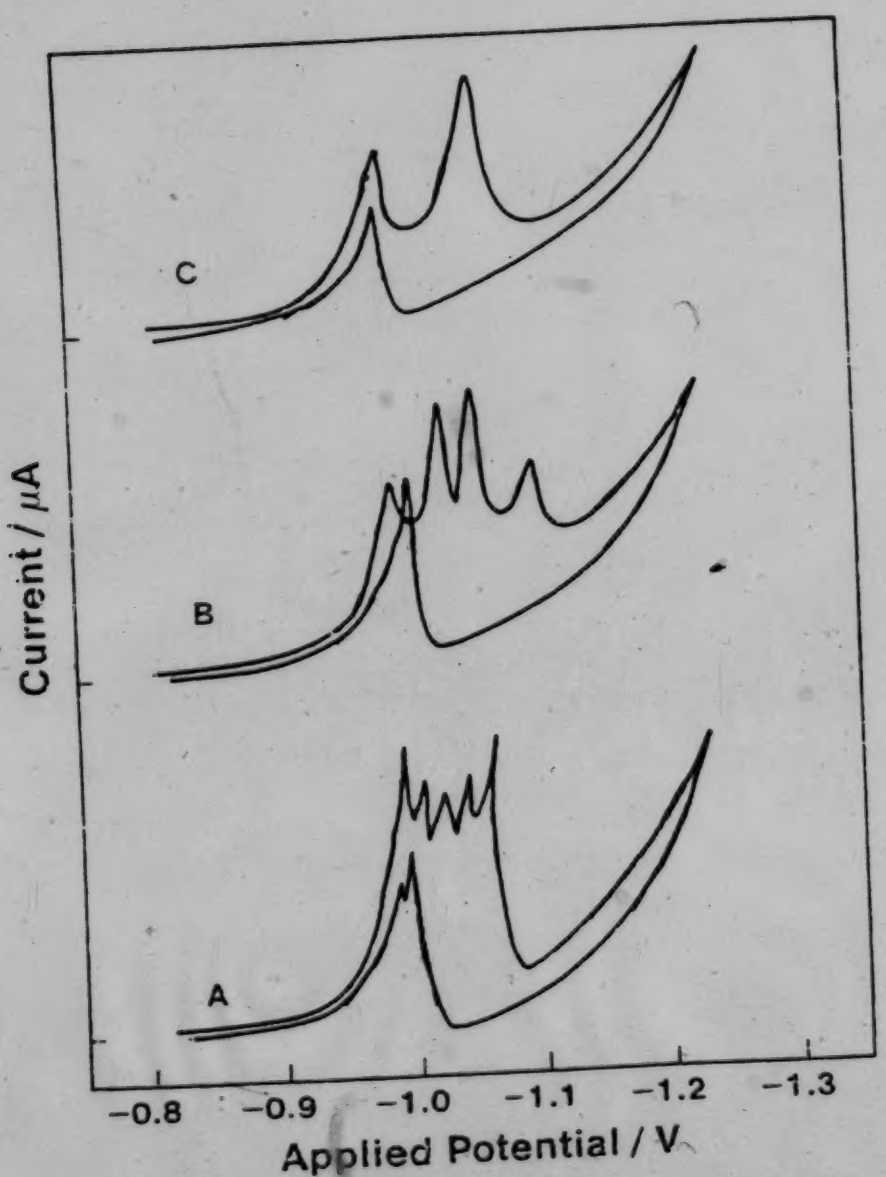


FIGURE 6: Cyclic Voltammogram of Minoxidil (5×10^{-4} M) in 1.0N H_2SO_4.

corresponding to the area under the peak is independent of sweep rate. In addition, ΔE_p is zero for pairs of adsorption - desorption steps or oxidation and reduction of absorbed layer.

Figure 5 illustrates the cyclic voltammogram of minoxidil (5×10^{-4} M) in dimethylformamide containing tetraethylammonium bromide. The Ep occurs at - 2.04V while the $E_{1/2}$ is at - 1.96V. At a scan rate of 20mV/s and a τ value of 10sec, the ratio of $I_{pa}/I_{pc}(37)$ is 1.09. The cathodic peak current decreases with decreasing scan rate but the graph of $I_p/\nu^{1/2}$ vs ν is non linear. The electroreduction process of minoxidil in the aformentioned system probably involved a process that is more complicated than one of simple diffusion. Strong multiple adsorption peaks, as illustrated in Figure 6, were observed in the cyclic voltammogram of minoxidil in 1.0N H_2SO_4. The peak centre occurs at about -1.0V, and the multiplicity of the peaks decreases with increasing scan rate.

2.8.10 Polarographic Behaviour of Pyrimidine and Some Selected Derivatives

Polarograms of pyrimidine and some selected derivatives were recorded for comparative purposes with that of minoxidil. The comparision will enable one to predict the nature of the polarographic waves expected for minoxidil and, ultimately to propose a mechanism for the reduction process.

2.8.10.1 Pyrimidine

As expected pyrimidine exhibited five polarographic waves over the pH range 0.5 to 11.0. The nature of the observed waves are consistent with those previously reported for pyrimidine (14). Figure 7 illustrates the sampled d.c. polarographic waves for pyrimidine over the pH range indicated. The electrochemical processes which resulted in these waves have already been discussed in the introduction.

2.8.10.2 2-Aminopyrimidine

Three polarographic waves were observed for this compound over the pH range 0.5 to 10.0. Only one pH-dependent wave was observed in highly acidic medium. This wave moves cathodically with increasing pH. At a pH of 4.0, a second wave emerges from the background discharge current. These two waves merge at a pH of about 7.0 to give the third wave. (see Figure 8). The inability of 2-aminopyrimidine to exhibit five polarographic waves is attributed to the presence of the amino group at position two of the pyrimidine moiety. Substitution at position two in pyrimidine makes the reduction of the 1,2-azomethine bond very difficult. The fourth polarographic wave of pyrimidine results from the reduction of the 1,2-azomethine bond (14). The reduction of 2-aminopyrimidine, therefore, involves only the 3,4-azomethine bond.

2.8.10.3 4-Methylpyrimidine

This compound exhibits all the polarographic waves observed for pyrimidine, except that the $E_{1/2}$ values are shifted to more negative potentials. This observation is consistent with the fact that the presence of the methyl group at position four makes the reduction of the 3,4-azomethine bond more difficult, hence the shift of $E_{1/2}$ values to more negative potentials. Figure 9 presents the sampled d.c polarogram of this compound over the pH range indicated.

2.8.10.4 2-Amino-4,6-dimethylpyrimidine

Only one polarographic wave was observed for this compound over the pH range 0.5 to 11.0. Theorically three waves should be observed, but because of the presence of electron releasing methyl groups at positions four and six, the intermediate radical formed as a result of a one-electron reduction of the 3, 4-azomethine bond to give wave I is very unstable and reacts rapidly at the potential of its formation to produce 3,4-dihydro-2- amino-4,6-dimethylpyrimidine. See Figure 10 for the sampled d.c. polarographic waves.

2.8.10.5 4-Amino-2,6-dimethylpyrimidine

Figure 11 illustrates the sampled d.c. polarographic waves for this compound. Its polarographic behaviour is similar to that of 2-amino-4,6-dimethylpyrimidine. The electroreduction process, however, might involve a

deamination step to give 2,6-dimethyl pyrimidine.

2.8.10.6 2,4-diamino-6-hydroxypyrimidine

This compound exhibits only one polarographic wave in highly acidic and moderately acidic media, pH range 0.5 to 5.0 with an $E_{1/2}$ value of -1.04V. The reduction process might involve only the 3,4-azomethine bond. The ease of the reduction is highly influenced by the hydroxy group at position six of the pyrimidine moiety. The hydroxy group can tautomerize to the keto form which is relatively difficult to reduce (Figure 12).

2.8.10.7 2,4-diamino-6-piperidinopyrimidine-3-oxide (Minoxidil)

Minoxidil exhibits two d.c. and d.p polarographic waves in 1.0N H_2SO_4 and in Britton-Robinson buffer pH range 3.0 to 7.0. In sulfuric acid, the first wave is intense and well-resolved from the second wave which is partially overlapped by the supporting electrolyte discharge current (Figure 13). At that pH, wave one had an $E_{1/2}$ value of -0.95V. In the Britton-Robinson system, however, the $E_{1/2}$ moves cathodically while the i_d decreases with increasing pH. The sampled d.c. polarographic waves are presented in Figure 14.

The second wave which has an $E_{1/2}$ value at approximately -1.200V has a diffusion current that is very much higher than that of the first. This unexpected height increase is the result of interferance by reduction of the supporting electrolyte. Both the i_d and $E_{1/2}$ of this wave vary with pH in

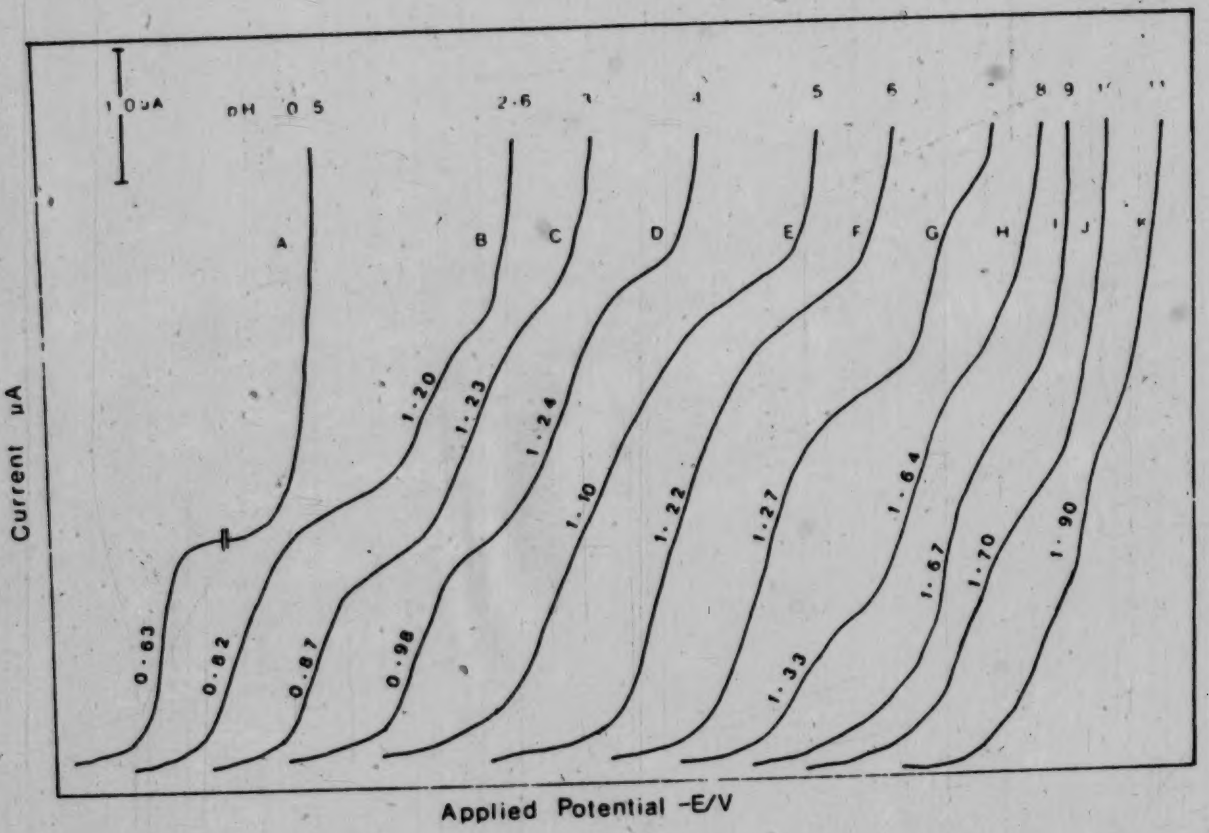


FIGURE 7: Effect of pH on the Sampled D.C. Polarographic Waves of Pyrimidine in A; 1.0N H_2SO_4, B - K; Britton-Robinson Buffer, pH Range 2.6 to 10.0. Numbers on Waves are the $E_{1/2}$ Values.

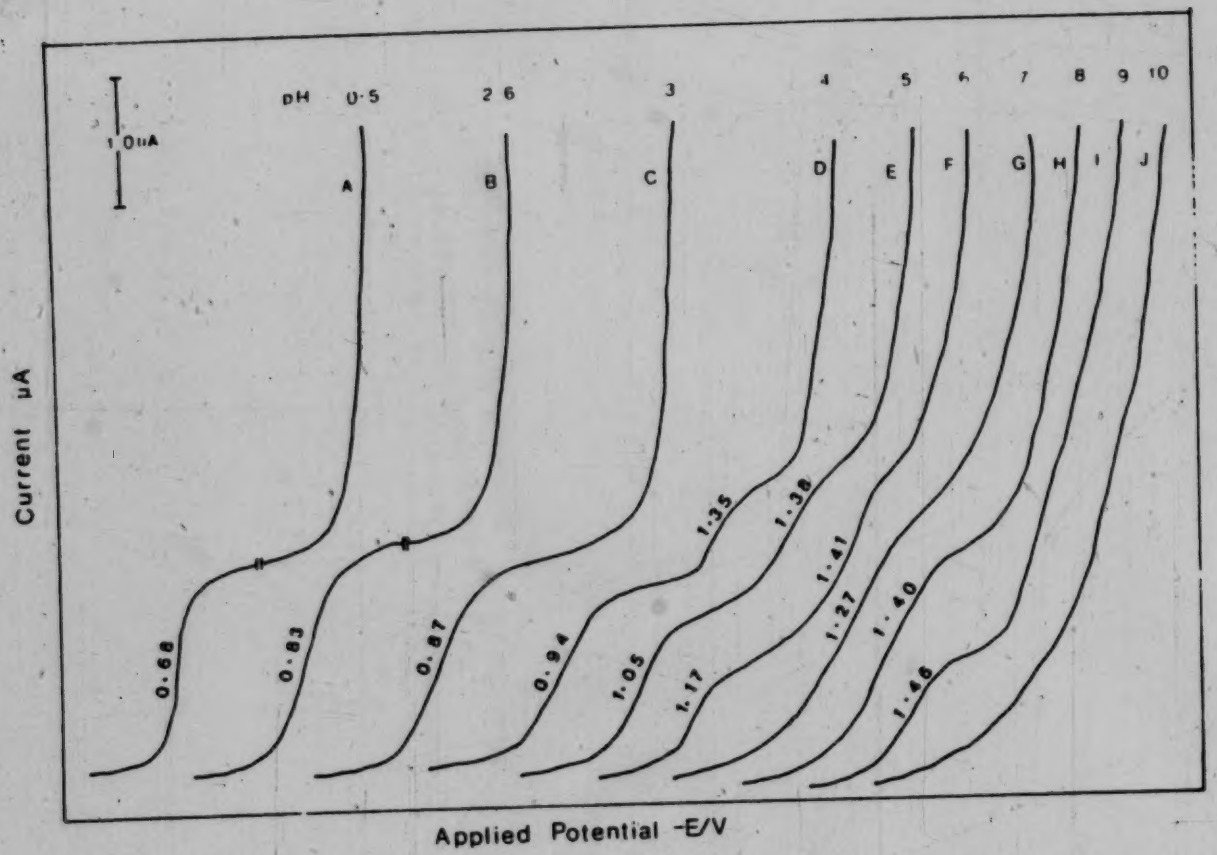


FIGURE 8: Effect of pH on the Sampled D.C. Polarographic Waves of 2-Aminopyrimidine in A; 1.0N H_2SO_4, B - K; Britton-Robinson Buffer, pH Range 2.6 to 10.0. Numbers on Waves are the $E_{1/2}$ Values.

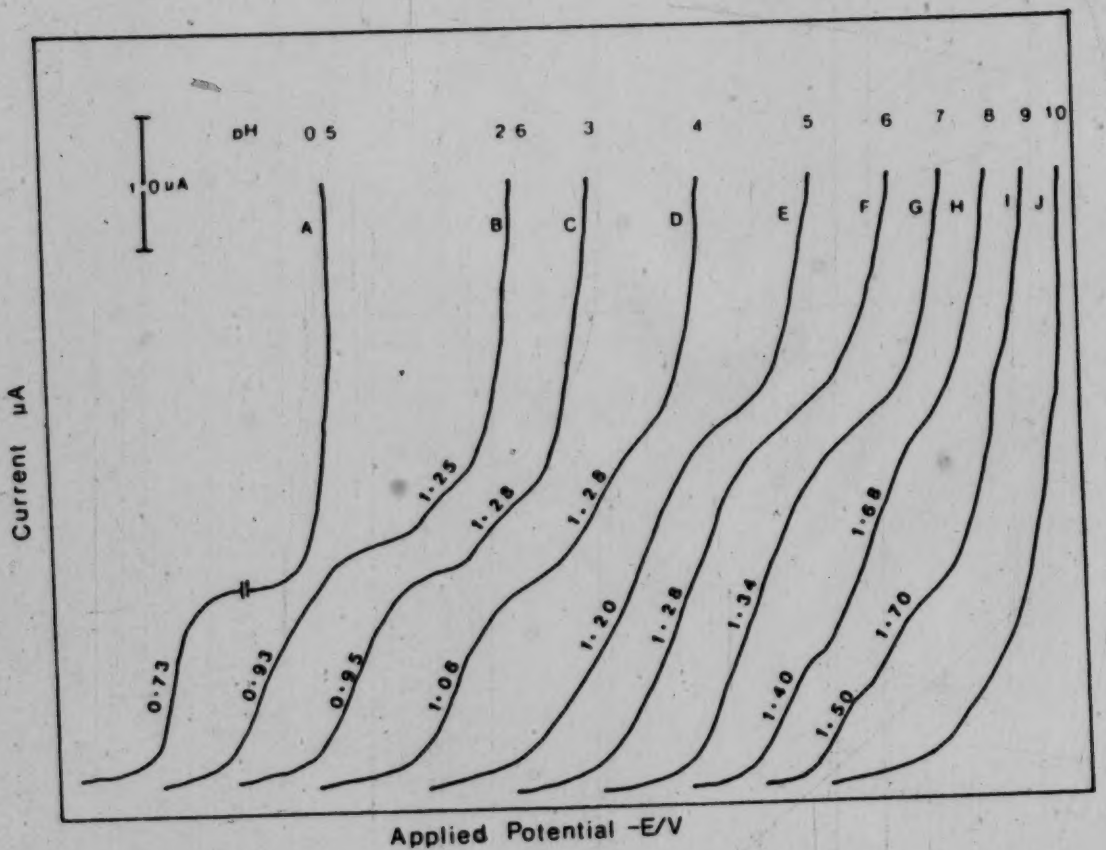


FIGURE 9: Effect of pH on the Sampled D.C. Polarographic Waves of 4-Methylpyrimidine in A; 1.0N H_2SO_4, B - T; Britton-Robinson Buffer, pH range 2.6 to 10.0. Numbers on Waves are the $E_{1/2}$ Values.

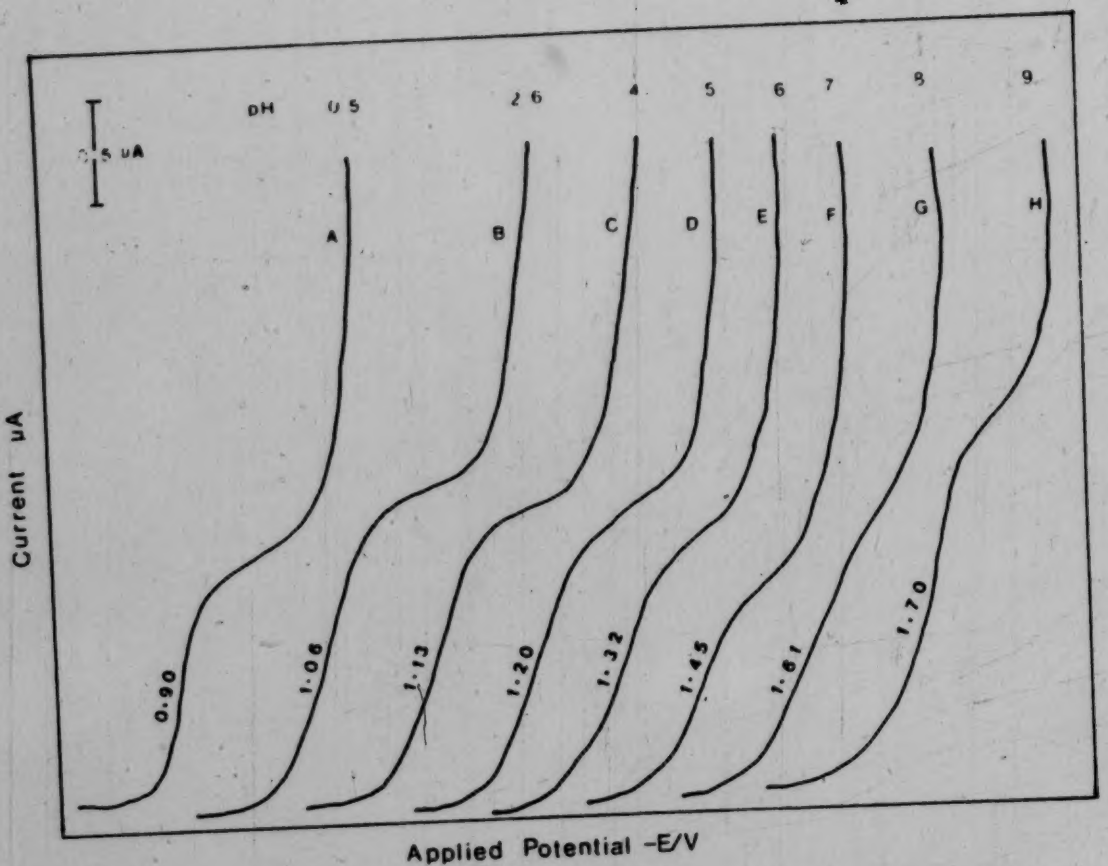


FIGURE 10: Effect of pH on the Sampled D.C. Polarographic Waves of 2- Amino-4,6-dimethylpyrimidine A; 1.0N H_2SO_4, B - H; Britton Robinson-Buffer, pH Range 2.6 to 9.0. Numbers on Waves are the $E_{1/2}$ Values.

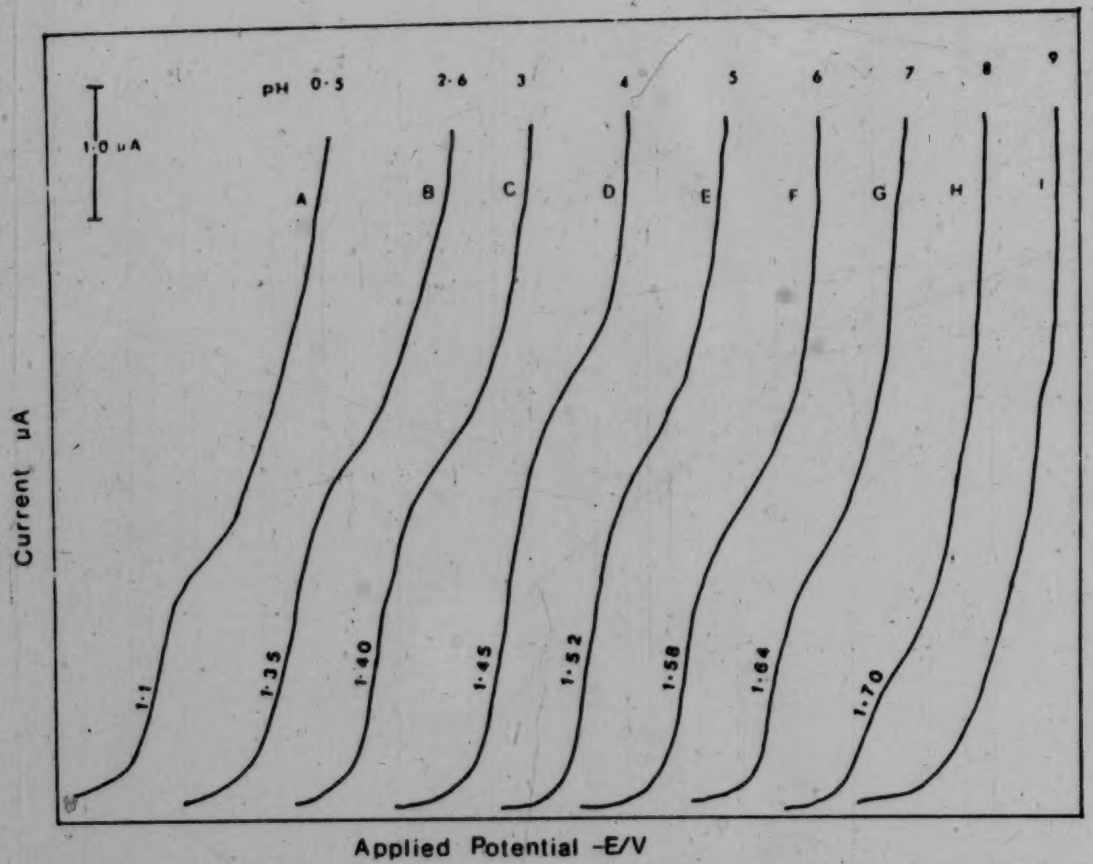


FIGURE 11: Effect of pH on the Sampled D.C. Polarographic of 4-Amino - 2,6-dimethylpyrimidine in A; 1.0N H_2SO_4, B - I; Britton-Robinson Buffer, pH Range 2.6 to 9.0. Numbers on Waves are the $E_{1/2}$ Values.

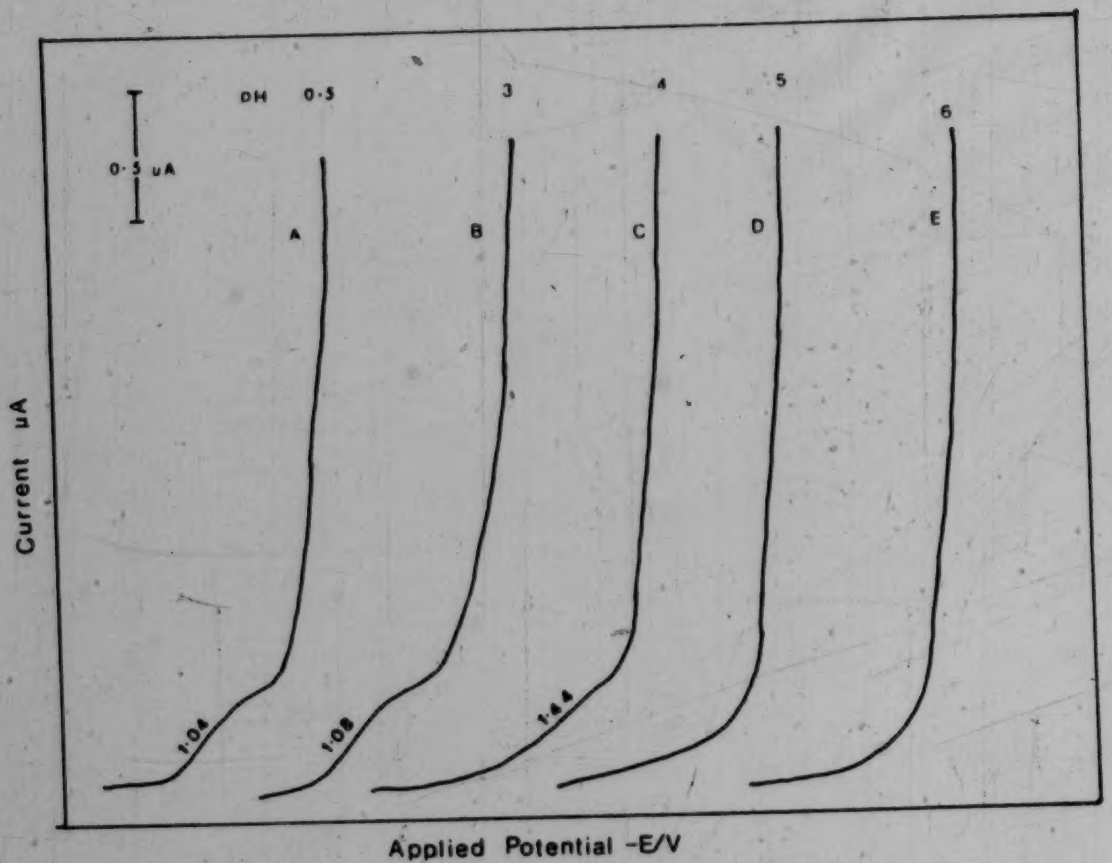


FIGURE 12: Effect of pH on the sampled D.C. Polarographic Waves of 2, 4 Diamino,6-hydroxypyrimidine in A; 1.0N H_2SO_4, B - E; Britton Robinson Buffer, pH range 3.0 to 6.0. Numbers on Waves are the $E_{1/2}$ Values.

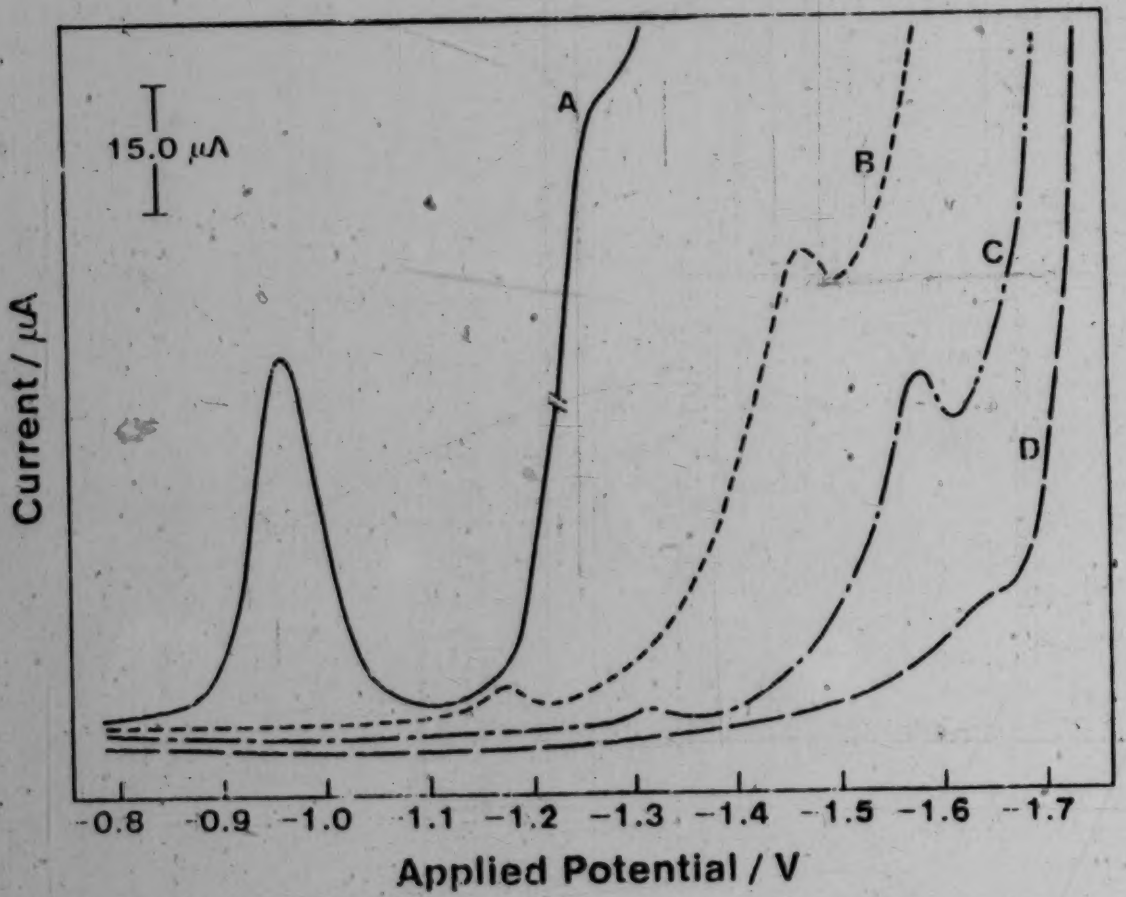


FIGURE 13: Effect of pH on the D.P. Polarographic Waves of Minoxidil (5×10^{-4} M) in A, 1.0N H_2SO_4 ,B,C and D Britton-Robinson Buffer, pH 3.0, 5.0 and 7.0 respectively.

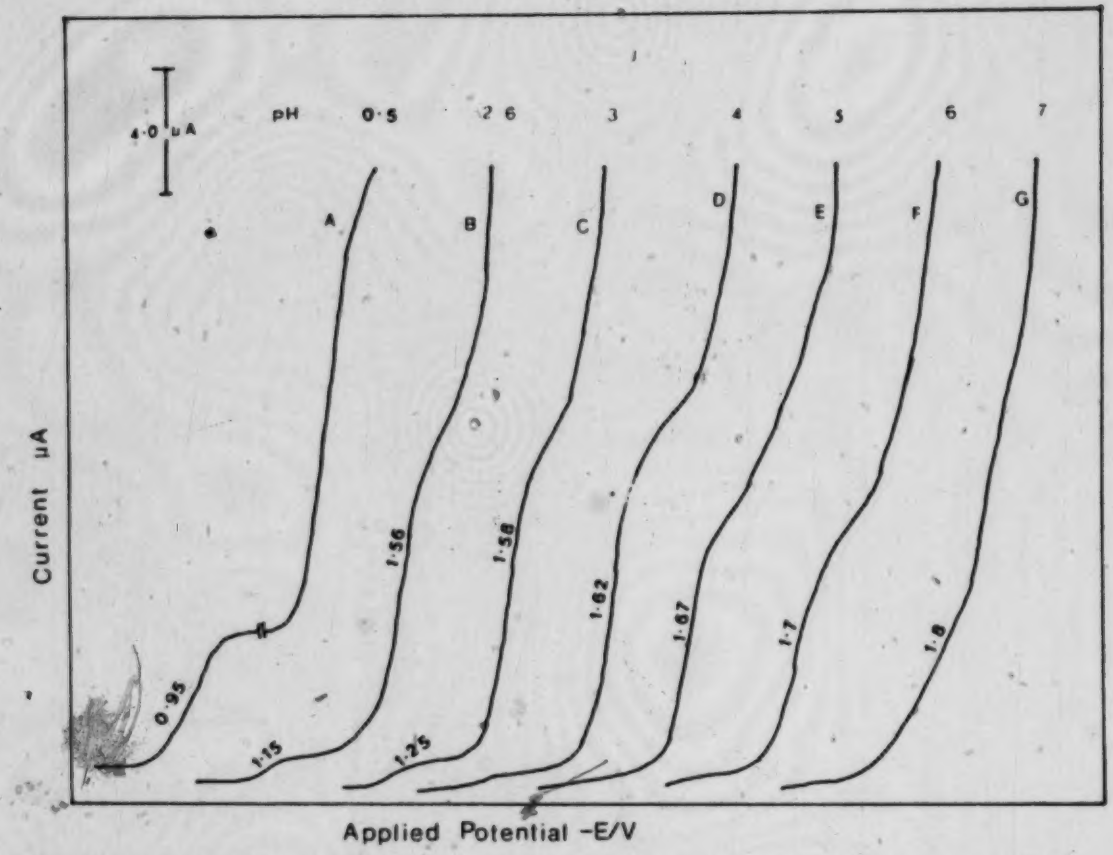


FIGURE 14: Effect of pH on the Sampled D.C. Polarographic Waves of 2, 4-Diamino,6-piperidinopyrimidine-3-oxide, (Minoxidil) in A; 1.0N H_2SO_4, B - G; Britton-Robinson Buffer, pH Range 2.6 to 7.0. Numbers on Waves are the $E_{1/2}$ Values.

the same manner as those of wave one.

The first wave is attributed to the reduction of the fully protonated N-oxide to an N-hydroxy intermediate which undergoes a two electron reduction to 2,4-diamino-6-piperidinopyrimidine intermediate. At higher potentials, the 3,4-carbon-nitrogen double bond can be reduced in a two-electron process to an unstable intermediate, 3,4-dihydro-2,4-diamino-6-piperidinopyrimidine. This intermediate undergoes deamination to give the final product, 2-amino-6-piperidinopyrimidine which was isolated in this work. The coulometric analysis of minoxidil in 1.0N H_2SO_4 indicates that four electrons per molecule were involved in the electroreduction process.

The major product extracted from the macroscale experiment emits a pinkish fluorescence under short UV light. It also produces a negative result with the ferric chloride test indicating the absence of an N-oxide group. The strong IR N-oxide absorption peak between 1,250 and 1,300/cm is absent in the IR spectrum of the product (Figure 15) as compared to that of minoxidil Figure 16. The NMR (200 MHz) spectrum of the product exhibits four distinguishable peaks in $CDCl_3$. In $CDCl_3$-D_2O system, however, the peak at δ 5.9 is reduced to a doublet with an integration value corresponding to one proton. No other change in the spectrum was observed (Figure 18).Figures 17-19 are the NMR spectra of minoxidil and reduced minoxidil.

FIGURE 15: Infrared Spectrum of reduced Minoxidil 2-Amino-6 piperidinopyrimidine

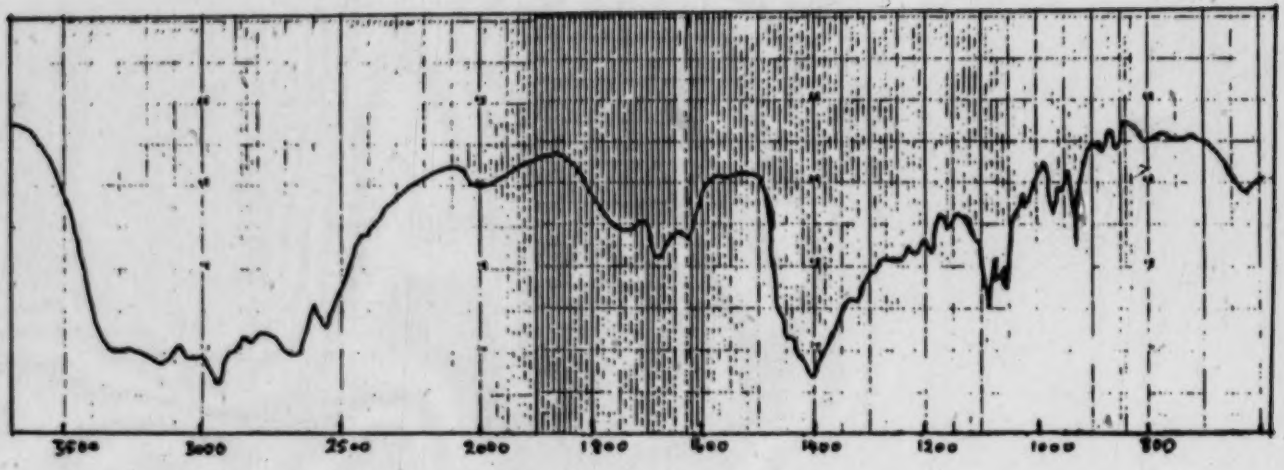


FIGURE 16: Infrared Spectrum of Minoxidil

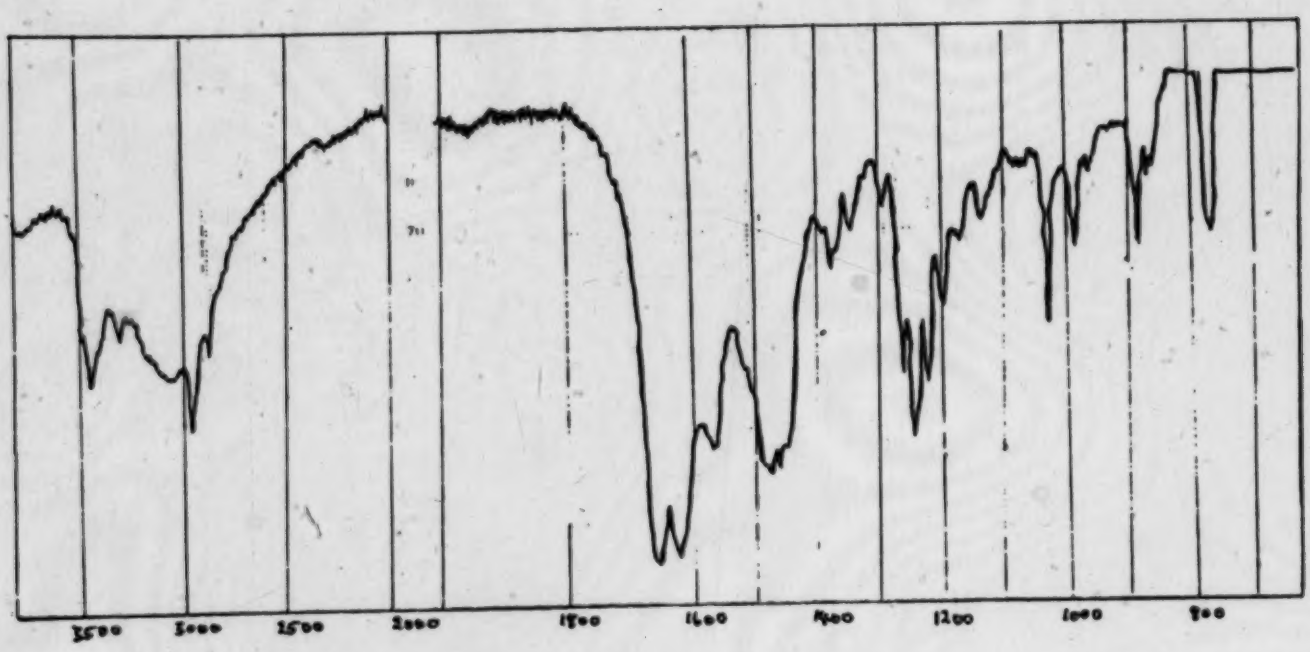


FIGURE 17 NMR Spectrum of Minoxidil in CD_3OD

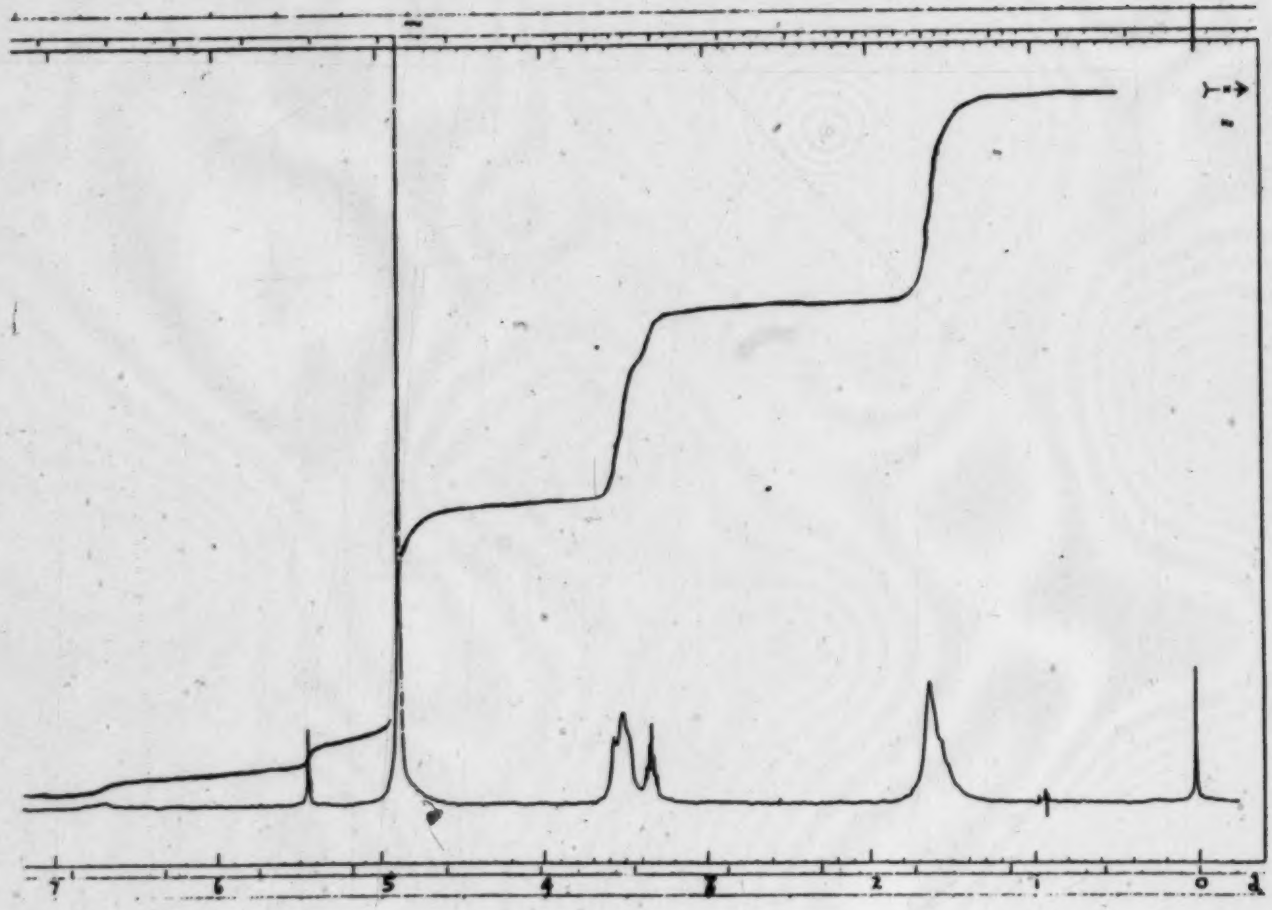


FIGURE 18: NMR of Reduced Minoxidil in $CDCl_3$

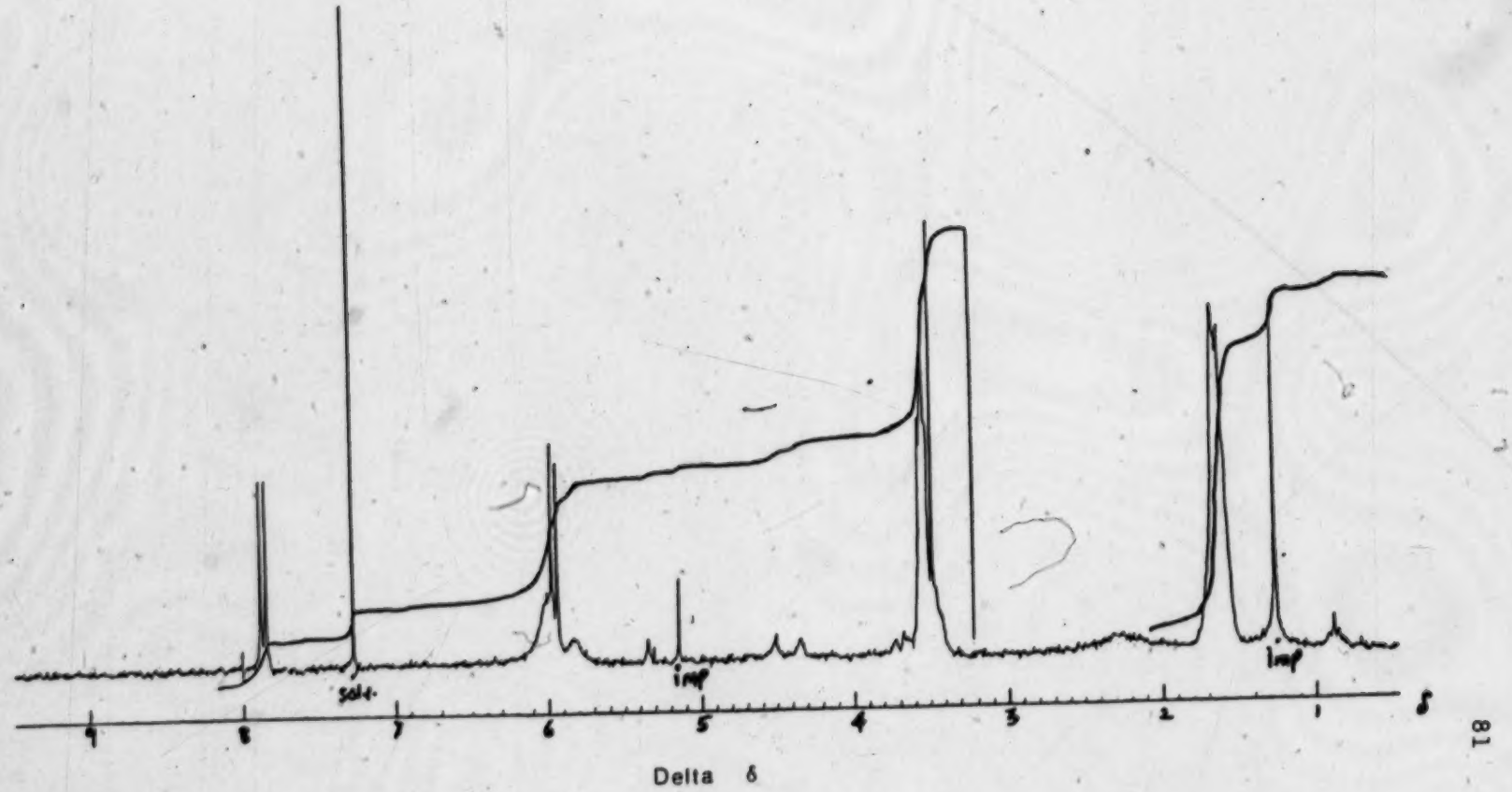


FIGURE 19: NMR of Reduced Minoxidil in $CDCl_3$-D_2O

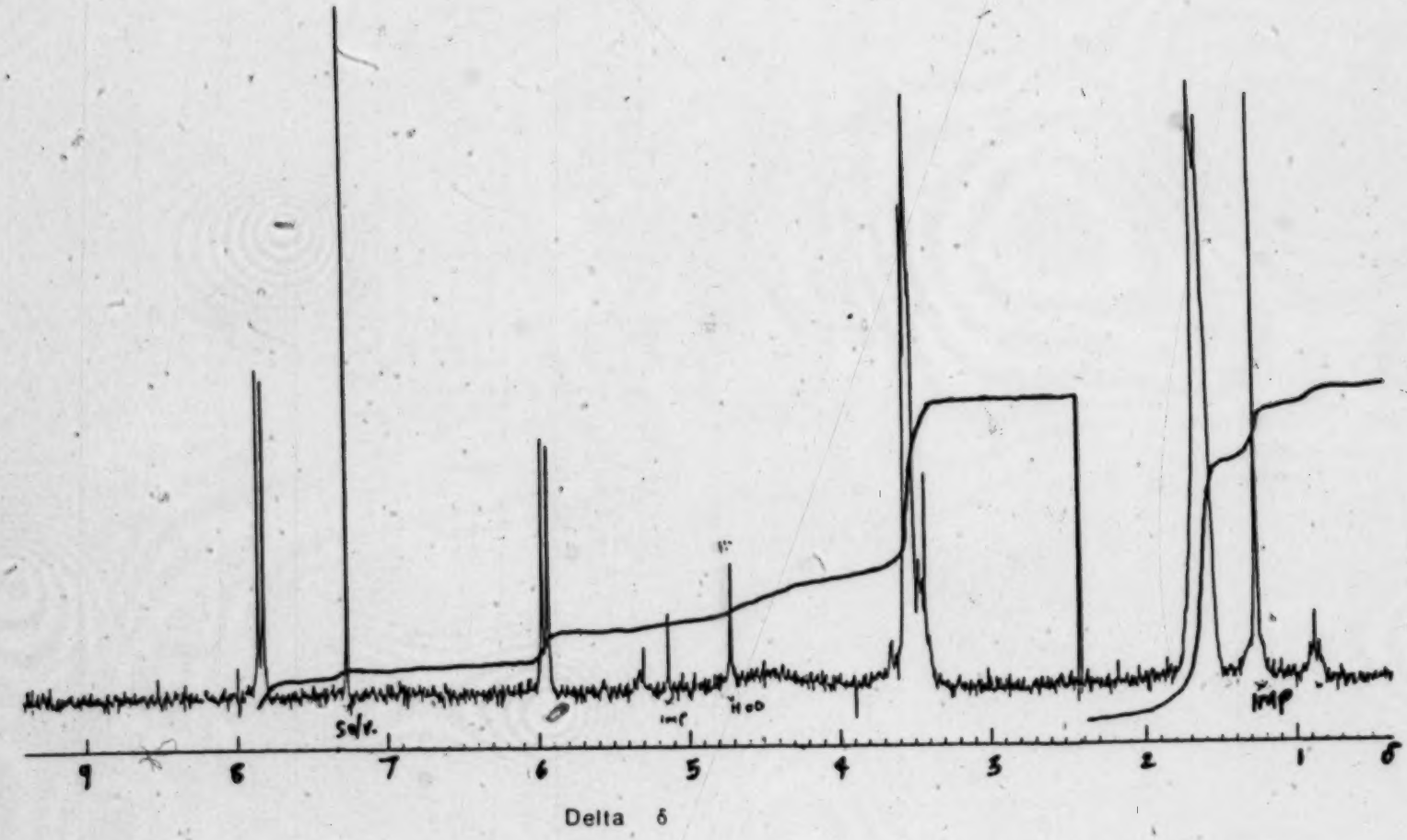


FIGURE 20: Mass Spectrum of Minoxidil

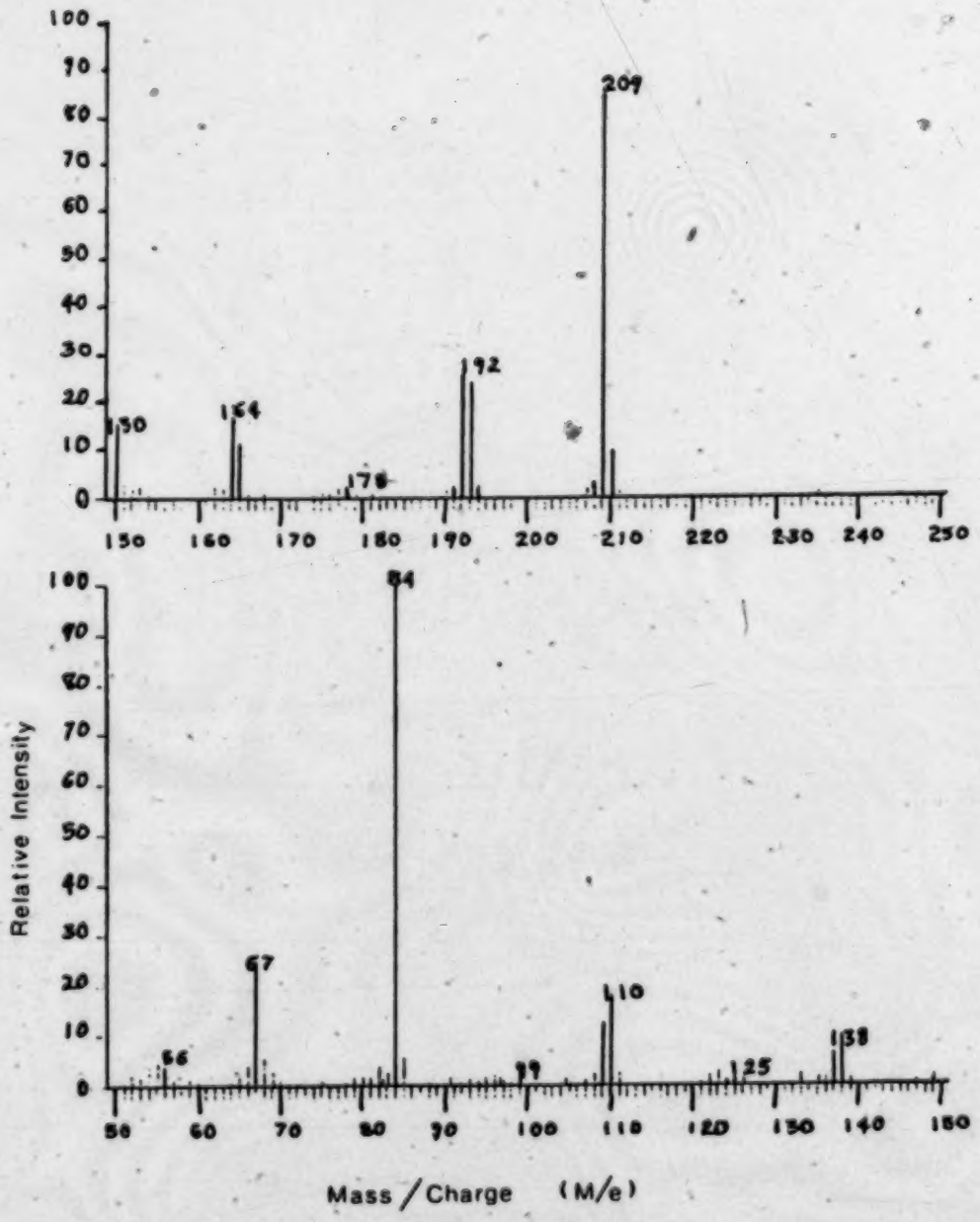


FIGURE 21: Mass Spectrum of Reduced Minoxidil

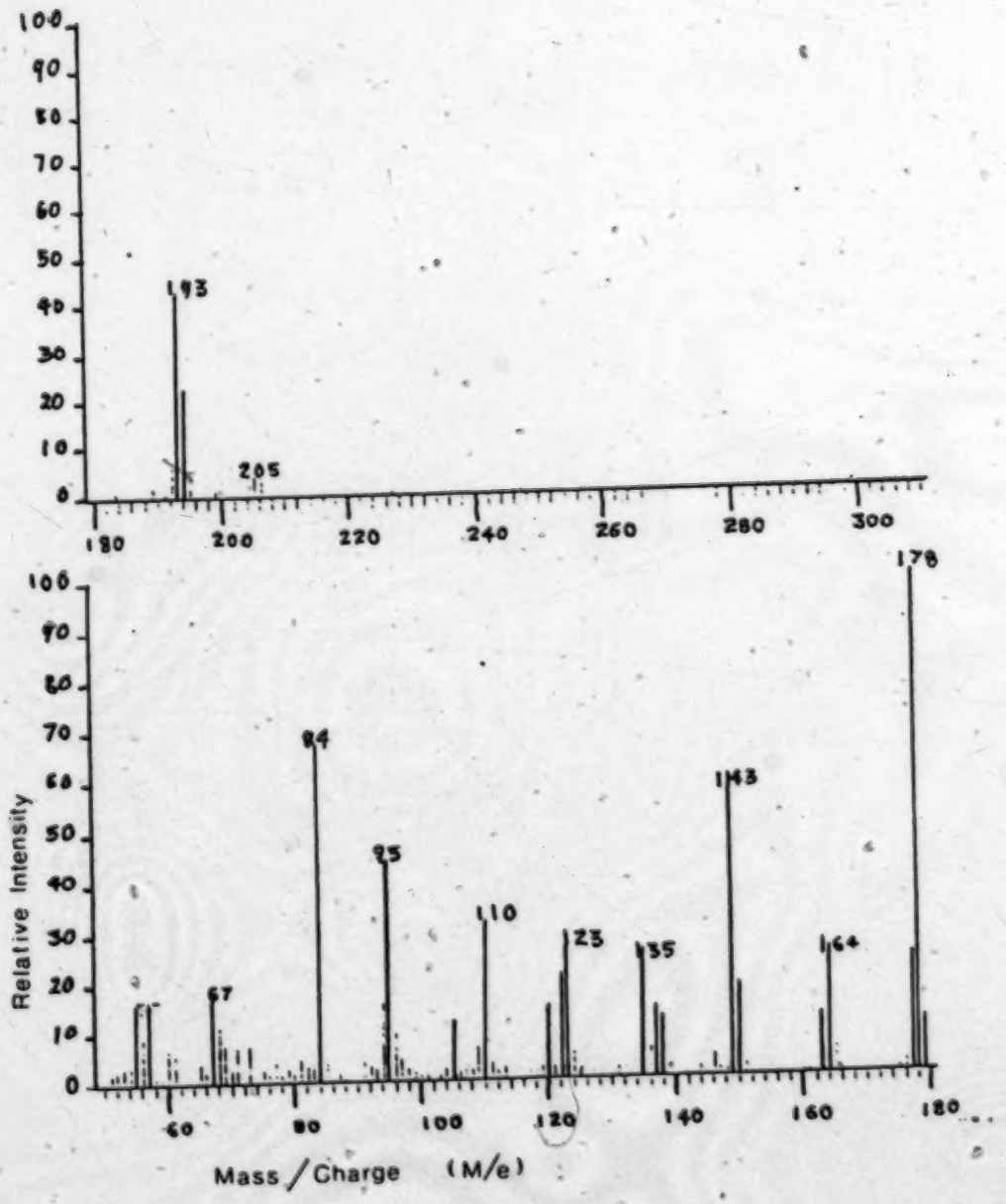


Figure 20 is the mass spectrum of minoxidil while Figure 21 exhibits that of the isolated major reduced product. The base peak is observed at m/e 178. Peaks with m/e values greater than 178 might come from fragmentation of higher molecular weight intermediates or impurities which might have been co-extracted with the major product.

From all the foregoing observations, the following pathway is proposed as minoxidil proceeds, by electrochemical reduction, to the final product, 2-amino-6-piperidinopyrimidine (Figure 22).

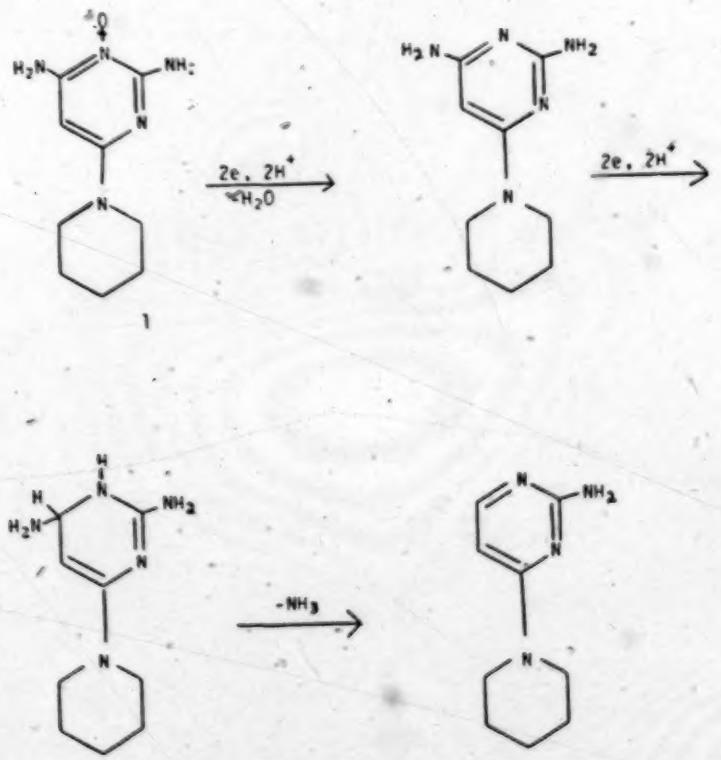


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Figure 22

2.8.11 Quantitative Assay of Dosage Forms

The proposed method was designed to overcome long extraction times and loss of material during the extraction step. Methanol was used as solvent for extracting the drug from the tablet mass because of the relatively poor solubility of minoxidil in water (1-3g/litre) whereas the solubility of minoxidil in methanol is between 10 to 20gm per liter. Reduction intermediates and products are also soluble in the methanolic system, consequently, a highly homogenous system was, therefore, maintained during the reduction process. Tablet excepients were not soluble to any detectable extent in methanol; as a result, an effective extraction was achieved. Minoxidil has also been found to be very stable in methanol.

In 1.0N H_2SO_4, the d.p. polarographic wave of minoxidil is better resolved and gives much higher sensitivity than does the d.c wave. Therefore, the differential-pulse mode was chosen as the most suitable for analysing pharmaceutical dosage forms. The d.p. polarographic peak current varied linearly with the concentration of the drug over the range 1×10^{-5} to 5×10^{-4} M. Tables 4 and 5 illustrate the actual d.p.p. data obtained for the analysis of 10 individual tablets taken from each dosage form, while Tables 6, 7, and 8 provide the results of the overall assay for each of the minoxidil dosage forms based on 20 tablets. Values obtained by the manufacturer's quality control laboratory are also presented for comparative purposes and excellent agreement

Table : 4

Data for Single Tablet Analysis of Minoxidil (2.5 mg/tab)

Tablet No	i (μA) 1	2	3	Average i (μA)	mg/tablet Recovered	% recovery	Average % recovery
1	0.76	0.76	0.76	0.76	2.46	98.7	
2	0.77	0.77	0.77	0.77	2.50	100.0	
3	0.74	0.74	0.75	0.74	2.41	96.5	
4	0.74	0.76	0.75	0.75	2.44	97.4	
5	0.73	0.76	0.75	0.75	2.44	94.7	99.88
6	0.78	0.78	0.80	0.77	2.55	102.0	
7	0.78	0.80	0.80	0.79	2.57	103.0	
8	0.80	0.79	0.79	0.79	2.57	103.0	
9	0.77	0.78	0.76	0.77	2.50	100.0	
10	0.81	0.75	0.78	0.78	2.53	101.3	

(1) Standard deviation = 0.06 mg.

Table : 5

Data for Single Tablet Analysis of Minoxidil (10 mg/tab)

Tablet No	i (μA) 1	2	3	Average i (μA)	mg/tab Recovered	%recovery	Average % Recovery
1	2.60	2.70	2.75	2.68	9.93	99.3	
2	2.65	2.65	2.70	2.69	9.94	99.4	
3	2.70	2.65	2.70	2.68	9.87	98.7	
4	2.78	2.75	2.75	2.76	10.23	102.3	
5	2.53	2.65	2.70	2.63	9.72	97.2	
6	2.65	2.75	2.63	2.68	9.93	99.3	100.71
7	2.70	2.68	2.70	2.69	9.97	99.7	
8	2.68	2.95	2.75	2.79	10.34	103.4	
9	2.75	2.75	2.90	2.81	10.40	104.0	
10	2.78	2.85	2.78	2.80	10.38	103.8	

(1) Standard Deviation = 0.74 mg

Table - 6

Data for Analysis of Pharmaceutical Dosage Forms.

Commercial Product	Manufacturer	Calculated Final conc $\times 10^{-3}$ M	Average Total current in μA	Found final conc $\times 10^{-3}$ M	%Labelled strength	Average % Recovery
Loniten 2.5mg/ Tablet	Upjohn	3.0	5.10	2.88	96.0	98.2 (2.4)mg
		3.0	5.23	2.96	98.6	
		3.0	5.15	2.92	97.3	
		3.0	5.35	3.05	101.0	
		3.0	5.20	2.95	98.3	
Loniten 10.0mg/ Tablet	Upjohn	3.0	5.12	2.90	90.6	96.6 (9.7)mg
		3.0	5.08	2.85	95.0	
		3.0	5.10	2.88	96.0	
		3.0	5.15	2.91	97.0	
		3.0	5.18	2.95	98.3	

Table : 7

Assay of Minoxidil Tablets by Differential-Pulse Polarography in a 1.0 N H_2SO_4-Methanolic System

Tablet Lot No	Label Claim /mg	Recovery by Manufacturer/mg	% Recovery by Manufacturer	Recovery by* D P Polarography/mg	%Recovered by D P Polarography
Lot H-651	2.5	2.49	99.6	2.46 + 0.09	98.2
Lot H-710	10	9.69	96.9	9.66 + 0.12	96.6

* Each value is the average of five determinations

Table : 8

Average Value for the Analysis of Ten Individual Minoxidil Tablets in A 1.0 N H_2SO_4-Methanolic System

Tablet Lot No	Labelled Claim/mg	Recovery by d.p.p./mg	% Recovery by d.p.p.
Lot H-651	2.5	2.49 + 0.1	99.9
Lot H-710	10	10.08 + 0.7	100.8

is observed between the two results. Common tablet excipients do not produce polarogaphic waves in the potential range investigated and they do not interfere with the method or cause any contamination of the electrode.

The procedures used for the analysis of dosage forms and for content uniformity test are similar except for differences in the initial treatment of samples before the extaction step.

The proposed method has the advantage of simplicity, high sensitivity and rapidity. It can detect minoxidil with a sensitivity of about 2mg/l. The developed d.p. polarographic method can distinguish between minoxidil and those degradation products that do not contain the N-oxide group and, consequently, it can be applied in purity and stability studies on minoxidil.

2.9 Summary and Conclusions

1) A simple differential-pulse polarographic method of analysis for minoxidil and its pharmaceutical dosage forms has been developed which is based on the electroreduction of the N -oxide and the 3,4-azomethine bond in the molecule. Excellent results were obtained when the method was applied to the analysis of tablets.

2) The actual mechanism of the electroreduction has been postulated. The reduced product was isolated and identified to be 2-amino,6-piperidinopyrimidine. Ultraviolet, infrared, nuclear magnetic resonance and mass spectroscopy were employed for the characterisation of the reduced product.

3) Studies on the effects of the composition of supporting electrolyte, the effects of pH of the system and on height of the mercury column have been conducted. The electroreduction has been confirmed mainly as diffusion dependent in the range of mercury column height and the concentration selected. From the studies it was found that the mercury column height of 75cm was suitable for each instance. 1.0N sulphuric acid was the most suitable system for the electro reduction. All measurements were made by employing a saturated calomel electrode (S.C.E.) as the reference electrode while a platinum wire was the auxiliary electrode. The $E_{\frac{1}{2}}$ was -0.95V.

4) A linear and reproducible calibration curve was obtained using pure reference minoxidil supplied by the

manufacturer. The d.p polarographic determination was based on a linear relationship between the diffusion current and the concentration of the species. A blank determination was required in each instance and the amount of minoxidil in each dosage form was calculated by the direct comparison technique. Commonly used tablet excipients were found not to interfere with the determination.

5) It is concluded that the accuracy, sensitivity, and reproducibility of this d.p polarographic method make it acceptable for routine analysis of minoxidil dosage forms. In addition, the method can be applied to stability studies on minoxidil.

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ARYL - ALKANOIC ACID ANTI-INFLAMMATORY AGENTS

3.2 Introduction

3.2.1 Non-steroidal Anti-Inflammatory Agents

The nonsteroidal anti-inflammatory agents (NSAIA) include some of the most widely used drugs in the world. Although their number is constantly being augmented by pharmacentical research, relatively few of the agents that have demonstrated anti-inflammatory activity are clinically useful.

Of the many conditions for which nonsteroidal anti-inflammatory agents are used, the rheumatic diseases are the most serious. The possible mechanism by which the anti-inflammatory activity of the drugs in this group is induced has been extensively discused (1). Most appear to act either by blocking the production or action of various local mediators of the inflammatory response. These include the polypeptides of the kinin system, the prostaglandins, which are becoming increasingly implicated as mediators of inflammation, lysosomal enzymes and the mediators of cellular activity such as the lymphokines. The systematic effects of the anti inflammatory agents, for example, their antipyretic action are probably a consequence of the blockage of the reactions in tissues that lead to the release of pyrogens.

Until recently, the most commonly used NSAIA's included the salicylates (Aspirin), acetophenetidin, acetaminophen, phenylbutazone and indomethacin. During the last decade a

new series of NSAIA's have either appeared on the market or are undergoing clinical trials all over the world. They are classified as the aryl-alkanoic acid type anti-inflammatory agents despite the fact that there exists certain structural differences among some of them.

Examples of NSAIA's already on the market include ibuprofen, tolmetin, ketoprofen and zomepirac sodium. Some of them have been found to be effective in the treatment of those types of mild and moderately-severe pain that previously had responded to treatment with steroidal anti-inflammatory agents. In some instances of pain not associated with an inflammatory condition ,they have been used to replace morphine.

As with many pharmacological agents, their usage frequently is associated with a number of adverse side-effects. In order to establish proper control, it is important, therefore, to investigate as many of their physico-chemical parameters as possible. Consequently, In this report, zomepirac sodium and ketoprofen have been subjected to polarographic investigation in order to determine their resulting products of reduction. An additional important objective is to develop electrochemical methods of assay for the two aforementioned anti-inflammatory agents as they occur in pharmaceutical dosage forms.

3.2.2 Zomepirac Sodium

Zomepirac sodium (sodium 5-(*p*-chlorobenzoyl)-1,4-dimethylpyrrole-2-acetate dihydrate) is a pyrrole acetic acid derivative structurally similar to tolmetin (2) (Figure 1). It is the first NSAIA to be approved for exclusive use as an analgesic for the treatment of moderately severe pain. It has been given the title "The first comprehensive non-addicting analgesic" by the manufacturer. Zomepirac sodium has the same basic pharmacologic action as other drugs of this group (ie, prostaglandin synthesis inhibition) and has been shown to inhibit prostaglandin synthesis, *in vitro* through inhibition of cyclo-oxygenase (2,3). Unlike the narcotic analgesics which act centrally, zomepirac sodium and other NSAIA's have a peripheral analgesic effect. Their analgesic effects result from decreased generation of prostaglandins which normally sensitize the pain receptors to the actions of other mediators. Zomepirac sodium, however, has high lipophylicity and this may explain some differences between it an other agents. Zomepirac sodium has also been shown to have antipyretic effects which in all probability are centrally mediated. It appears that this analgesic would have its greatest efficiency in conditions in which pain is closely tied to inflammation. Zomepirac sodium is readily absorbed from the gastro-intestinal tract producing peak plasma concentration within 1 to 2 hrs. It is reported to have a plasma half life of about 4 hrs, and to be extensively bound to plasma protein. The drug is excreted

in the urine mainly as the glucuronide (4).

A preliminary report summarising the adverse effects observed after multiple-dose administration of zomepirac has appeared (5). The most common adverse effects reported were gastrointestinal, urogenital symptoms, tinnitus and hearing loss. In a report on the long term effects of zomepirac by Honig (5), increases in BUN and creatinine compared with base line values were oberved.

Peripheral edema has also been noted in some patients. Contraindications for the use of zomepirac sodium include patients who have previously exhibited intolerance to it. Because it is tumorigenic in rats, the drug is considered to be contraindicated in children, pregnant women, and nursing mothers (6).

The recommended oral dose of zomepirac sodium is 100mg every 4hrs. In mild pain, 50mg (one half tablet) every 4 to 6hrs may be adequate (6). During the course of this work, zomepirac sodium has been withdrawn from the market owing to reports that the drug has been implicated in five deaths.

Zomepirac sodium is synthesized by a seven step reaction sequence starting with diethyl acetone dicarboxylate and aqueous methylamine (a modification of the Hantzch pyrrole synthesis) (7). The drug is an off-white powder which melts with decomposition at 178-179°C. The sodium salt is a yellow crystalline powder which is soluble

in water and methanol.

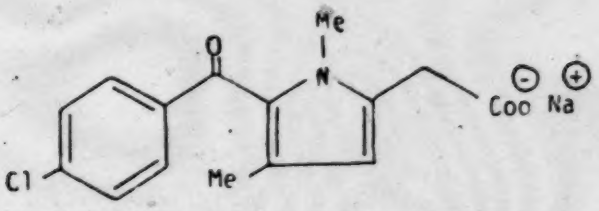


Figure 1. Zomepirac Sodium

3.2.3 Ketoprofen

Ketoprofen belongs to the group of new non-steroidal anti- imflammatory drugs, which have been developed during the past few years. The chemical composition of ketoprofen is 2-(3-benzoyl.phenyl)-propionic acid. Owing to an asymmetric carbon atom, ketoprofen is a racemic compound from which both the dextro and levo rotatory compounds have been isolated. The compound is a white, odourless, sharp-bitter tasting, crystalline powder. It is non hygroscopic in a relative humidity of 70%. Ketoprofen is soluble in benzene, ethanol, chloroform, acetone, ether and in alkaline solutions, but is practically insoluble in water.

Ketoprofen has been found to have analgesic, anti-inflammatory and antipyretic activities and is currently used for the treatment of rheumatoid arthritis and osteoathritis (8). The compound resembles naproxen, ibuprofen, and tolmetin in structure and activity. It

inhibits prostaglandin synthesis. Ketoprofen is readily absorbed from the gastrointestinal tract; peak plasma concentration occurs one half to one hour after an oral adminstration and the plasma half life is between 1 to 4 hrs. Ketoprofen and its metabolites are excreted mainly in the urine. Excretion has been found to be complete by the fifth day after administration. The drug binds extensively to plasma protein, and it is excreted mainly as a glucuronide conjugate The most frequent adverse effects occurring with ketoprofen are gastrointestinal disturbances, peptic ulceration and gastrointestinal bleeding.

Hypersensitivity reactions, abnormalities of liver function tests, impairment of renal function, agranulocytosis, and thrombocytopenia have occasionally been observed (9). Ketoprofen has no effect on drug metabolising enzymes in the liver, and is unlikely to produce clinically significant interactions with other drugs metabolised in the liver (10).

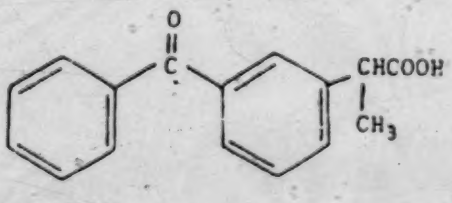


Figure 2. Ketoprofen

3.3 Literature Survey

Several methods have been reported in the literature for the determination of some of the newly developed aryl-alkanoic acid type non-steroidal anti-inflammatory agents. Most of the described methods involve the determination of these compounds in biological fluids (blood and urine). The analytical techniques described for the determination of these drugs involve T.L.C., non-aqueous potentiometric titration, colorimetry and polarimetry, G.L.C., H.P.L.C., radioimmunoassay, spectrofluorimetry, UV spectrophotometry and polarography.

The official methods for the identification and assay of zomepirac sodium in the pure form or in pharmaceutical dosage forms involve UV spectrophotometry, T.L.C. and non-aqueous Potentiometric titration (11). The non-aqueous titrimetry with a potentiometric end-point detection involves the titration of zomepirac sodium with perchloric acid in glacial acetic acid. This method is simple but lacks specificity since the presence of any other proton accepting species / or decomposition product of zomepirac can greatly interfere with the method. The T.L.C. procedure only lends itself to qualitative determination besides the fact that decomposition products which have the same R_f values as zomepirac sodium cannot be detected. The assay method outlined for dosage forms involves three extractions and two centrifugation steps prior to the final UV spectrophotometric analysis at 329 nm. The method is tedious

and requires very careful sample handling which is not usually amenable to routine analysis.

For analysis of biological fluids, colorimetry has been found to be applicable to both plasma and urine investigations, while polarimetry is only suitable for analysis of urine. The limit of sensitivity of the colorimetric method is 2-5mg/l and for the polarimetry 5mg/l (12).

Gas-liquid chromatography (13) is a more complicated method which requires extensive sample preparation and usually includes a derivatization step. For example, carboxylic acid molecules have to be converted to their corresponding methyl esters before injection into the column. The G.C. technique is, however, sensitive and can be used for analysis of serum and urine samples. The lowest limit of sensitivity for ketoprofen is 0.02-0.04mg/l.

Mitchell *et al* (14) used a modified gas liquid chromatographic method to determine ketoprofen in both plasma and synovial fluid. Populaire *et al*. (12) have described a polarographic technique for the determination of ketoprofen in urine. The method involves an extensive sample treatment since other urine components interfere with the method. The procedure used 0.2M tetrabutylammonium hydroxide as the supporting electrolyte while the instrument was operated in the alternating current mode. The applied potential range was -1.0 to -2.0 volts, and the $E_{1/2}$ value of the analytical wave occurred at -1.36 volt. The method is claimed to be

easy and rapid but at certain concentrations of urine there was an imprecision in the method owing to irregularities in the reduction of the carbonyl group because of the presence of some urine constituents. A radioimmunoassay method for the determination of ketoprofen and its metabolities in biological fluids has been described by Delbarre *et al*.(15) which involved labelling the methyl side chain with tritium. The samples were counted by a liquid scintillation apparatus. The method has been found to be more sensitive than the colorimetric, chromatographic and polarographic methods used for following the pharmacokinetics of ketoprofen.

Ko *et al*.(16) have developed an H.P.L.C. procedure for the determination of the purity of zomepirac sodium. The method can separate and quantitatively determine the synthetic impurities and degradation products from the drug substance. In addition, it does not generate any impurity or degradation products.A Micro silica gel column was used while two solvent systems (eluent) connected in the gradient elution mode were employed. The solvent systems were 0.25% acetic acid in *n*-hexane; and 0.25% acetic acid and 10% propan-2-01 in hexane. The second solvent was varied from 3% to 90% over 20min. The effluent was monitored by UV detector.

Kung-Tat *et al*.(17) have also developed an H.P.L.C. method for the the determination of zomepirac in plasma. The method is claimed to be simple, specific, sensitive and it

can detect the drug to about 10µg/ml with an ultraviolet detector set at 313nm to monitor the effluent. The stationary phase was a 10µm Lichrosorb Si60 while the mobile phase was composed of hexane, isopropanol and glacial acetic acid in the ratio 93:2:5 v/v.

A number of other methods have been reported for the determination of the aryl-alkanoic acid type anti-inflammatory drugs in serum. These include colorimetry, spectrofluorimetry, gas chromatography and radioimmunoassay. Some of these methods are non-specific while others involve complex extraction procedures and derivatization steps.

Ballerini *et al*.(18) employed a T.L.C. technique to determine ketoprofen in body fluids. The method is based on a quantitative ether separation, spot visualization, elution, and determination at 255nm. They claim the acid is detectable in amounts as low as 10ng. The chromatograms were developed in an ascending system of ether: benzene:1-butanol:methanol (85:8:6:1) in a saturated atmosphere. Visualization of spots was by exposure to UV lamp.

Numerous other H.P.L.C. methods for the determination of ketoprofen in body fluids have appeared in the literature during the past half decade. Upton *et al*.(19) reported an H.P.L.C. method that permits the convenient and rapid assay of ketoprofen and naproxen in biological samples at a sensitivity (10 and 2ng/ml, respectively in plasma; 20 and

50ng/ml in urine) far greater than previously available. They attributed the superior sensitivity to the buffered neutral eluent they used, which yields improved separation from materials of biological origin. There were no interferences from the major ketoprofen and naproxen metabolites tested and excellent reproducibility and accuracy could be maintained. The method shows promise of applicability to ibuprofen, fenoprofen and other members of the aryl-alkanoic acid class of NSAIA. The apparatus consisted of a pre-column containing handy pack VYDAC 30-44μm particles 3.2x40mm and an analytical column containing spherisorb ODS, 5μm particles 4.6x40mm. The eluent was a phosphate buffer (0.05M) pH 7.0 containing 6-8% acetonitrile, and maintained at a flow rate of 2ml/min. Assay time was 15 to 20 min.

Dusci *et al.*(20) have also reported an H.P.L.C. technique for the determination of some of the new aryl-alkanoic acid type NSAIA's in serum. The method requires only small samples of serum and an identical protein precipitation extraction procedure with acetonitrile. The method can also be used for screening in cases of overdosage of these drugs. A variable wave length UV detector was used while the column was a 30cm x 309mm. tube packed with μ Bondapack C (WA). The elution solvent was 60% acetonitrile in 45mM KH_2PO4 adjusted to pH 3.0 with orthophosphoric acid.

A flow rate of 0.8ml/min was employed while the effluent was monitored at 225nm. Some of the anti-inflammatory agents assayed by this method include oxyphenbutazone, indomethacin, phenylbutazone, mefenamic acid, fluternamic acid, ibuprofen and naproxen. Jefferies *et al*.(21) claim to have developed an H.P.L.C. method that requires minimum sample preparation and a minimum quantity of ketoprofen, for example 2.5mg can be determined in an analytical time of 4 min. The extraction step involved a hydrolysis step for the glucuronide conjugate of the drug. This step is ommitted if only free ketoprofen is present. The column was a 50 x 4.6mm slurry-packed with spherisorb 5-ODS 5µm particles while the eluent was 35% methanol in distilled water at pH 3.5 with acetic acid at a flow rate of 2ml/min.

In another report, Bannier *et al*.(22) compared results obtained by a developed H.P.L.C. method with a G.C. method. The developed method involved extraction and methylation with gaseous diazomethane before the H.P.L.C. analysis. The column used consisted of LiChrosorb Si60 (5µm) and a dichloromethane:hexane (60:40) solution as the mobile phase. The precision of the method was ± 4% and the lower detection limit ranged from 0.06 to 0.1µg/ml. Results obtained by the H.P.L.C. method showed very good correlation with those obtained by gas-liquid chromatography.

The only method of analysis that is available for some of these aryl-alkanoic acid analgesics appears to be the

official UV spectrophotometric procedure, since no other assay has been reported in the literature. Structural examination of these substances reveals a common feature which could be utilized for their assay. Most of them contain a benzoyl group attached to an aromatic ring. Consequently they are classified as phenylketones, and can be amenable to voltammetric analysis. The simple electrochemical behaviour of the carboxyl group has been utilized by various workers for the assay of ketone based compounds, eg.; the butyrophenones. Volke *et al*.(23) have determined haloperidol and two related *p*-fluro substituted butyrophenone derivatives, trifluperidol and fluanison by direct current oscillographic polarography. Concentrations from 10^{-3} to 10^{-5} M of each of the compounds were determined in a system of 0.25N potassium hydroxide in 50% ethanol, and in pH 5.3 acetate buffer in 50% DMF. Mikolajeh *et al*.(24) have recently described a rapid and simple procedure for the determination of the toxicity levels of trifluperidol and haloperidol in blood. The assay involved selective extraction of the compounds from alkalinized blood into cyclohexane, the extract concentrated to dryness and then dissolved in Britton-Robinson buffer, pH 4.6, containing methanol (2:3v/v) for polarographic analysis. The assay has a sensitivity of 10mg/ml with a relative standard deviation of ±4%.

3.3.1 Statement of Purpose

The main objectives of this present work are : To investigate the polarographic behaviour of some of the aryl-alkanoic acid analgesics such as zomepirac sodium and ketoprofen. To isolate and characterize the electrochemical reduction products of these compounds and finally, to develop a polarographic method for the determination of these compounds in pharmaceutical dosage forms.

To accomplish the objectives outlined, some variables (with respect to these compunds) to be investigated include :

i) The search for an optimum supporting electrolyte system.

ii) The effect of pH on the $E_{1/2}$ values of the polarographic waves exhibited by the compounds.

iii) To determine the diffusion dependency by plotting the diffusion current versus the square root of the corrected height of mercury column.

iv) To investigate the electrochemical behaviour of the compounds by cyclic voltammetry and to determine the effect of scan rate on their cyclic voltammetric peak height.

v) To determine the number of electrons transferred per molecule during the electroreduction process.

vi) To perform macroscale electrolysis of these compounds.

vii) To propose a mechanism for the reduction process.

viii) To prepare a calibration graph and to determine the coefficient of correlation between peak current and concentration of the electroactive species.

ix) To apply the developed method to the analysis of pharmaceutical dosage forms.

EXPERIMENTALS

3.4.1 Apparatus and Conditions for Polarographic Analysis

Standard laboratory glassware, one meter rule, stop watch, nitrogen tank, Mettler balance, magnetic stirring device. Unicam SP 1800 UV spectrophotometer and recorder. Perkin-Elmer 267 IR spectrophotometer Unicam SP 1000 IR spectrophotometer, 200MHz NMR spectrometer, 60MHz NMR spectrometer. Other apparatus will be discussed under appropriate sections.

Reference Standards.

i) Zomepirac sodium (99.44%) was obtained from McNeil laboratories, Canada, Ltd.

ii) Deschlorozomepirac obtained from McNeil Laboratories

iii) Ketoprofen (99.8%) obtained from Rhone-Poulenc Pharma Inc. Canada. Ltd.

Reagents

The following reagents were used, all of analytical-reagent grade: dimethylformamide, barbital, boric acid, citric acid, lithium perchlorate, potassium dihydrogen phosphate, anhydrous methanol, 0.2N sodium hydroxide, tetraethylammonium bromide, 0.2N HCl and 1% lithium perchlorate in DMF. Britton-Robinson buffers were prepared with distilled and deionized water at intervals of 0.2-0.3pH unit over the pH range of 6.0-11.0.

Pharmaceutical Dosage Forms

Compound Trade Name	Manufacturer	Dosage Form	Lot No.
Zomepirac sodium (Zomax)	McNeil Labs. (Canada) Ltd.	100 mg Tablets	Dj-3187 Mc-4782
Ketoprofen (Orudis)	Rhone-Poulenc Pharma Inc. (Canada) Ltd.	50 mg Capsules	247
Ketoprofen (Orudis)	Rhone-Poulenc Pharma Inc. (Canada) Ltd.	100 mg Suppositories	169

3.4.2 Procedure

3.4.2.1 A) Determination of Reference Standard Purities

The purities of reference standards of zomepirac sodium, deschlorozomepirac and ketoprofen were checked by means of thin layer chromatography. Eastman Chromatogram silica gel with fluorescent indicator plate was used while

the solvent system was chloroform:glacial acetic acid(90:10).

3.4.2.2 B) Constant Temperature Control for Polarography

The polarographic cell was immersed in a water bath connected to a thermostatically controlled reservoir. Circulation was ensured by means of a pump. The temperature of the water was maintained at a constant value of 25 ± 1°C.

3.4.2.3 C) Determination of Optimum Height of Mercury Column

Zomepirac sodium (5×10^{-4} M) was used for determining the optimum height of mercury column. This solution was prepared by diluting 1ml of 10^{-3} M methanolic stock solution of zomepirac with 19ml of Britton-Robinson buffer, pH 11.0. After deaeration for 10 min with purified nitrogen the polarograms were recorded at various heights of the mercury column ranging from 60cm to 80cm with 5cm as the interval. The dropping time was maintained at 2s by an automatic drop timer. Comparisons of the polarograms were made and the optimum height of mercury column was selected. The same height was used for the studies on ketoprofen.

3.4.2.4 D) Determination of Optimum Buffer or Supporting Electrolyte System

Britton-Robinson buffer was chosen as the supporting electrolyte system for the investigation. The pH range was 6.0 to 11.0 because highly acidic media resulted in precipitation of zomepirac as the free acid.

Working solutions (5×10^{-4} M) of zomepirac sodium and ketoprofen were prepared individually by diluting 1ml of each stock solution of zomepirac sodium and ketoprofen (10^{-2} M) with nineteen ml of the buffer. Polarograms were recorded for each substance after deaeration for 10 min with nitrogen. For zomepirac sodium, the pH of the buffers was varied form 6.0 to 11.0, at intervals of 0.2 to 0.3 pH units. The intervals for ketoprofen were 1 pH unit. A comparison of polarograms obtained for each compound was made in order to choose the optimum pH for the analysis.

3.4.2.5 E) Determination of Solution Stability

The stability of stock solutions of zomepirac sodium and ketoprofen were determined by comparing polarograms obtained with a freshly prepared solution to those obtained with a five day old solution of each substance.

3.4.2.6 F) Determination of pH Change Before and After Electrolysis

The pH values of the solution were measured electrometrically prior to and after the polarographic reduction, in order to ensure that the pH had remained relatively constant.

3.4.2.7 G) Apparatus and Conditions for Polarographic Analysis of Zomepirac Sodium

A Fisher, Model 320, pH meter fitted with a glass-calomel electrode system was employed to measure the pH values of the solutions.

A PAR, Model 174, polargraph equipped with a drop timer (Model 172A) and a Houston Omnigraphic Model 2000 recorder were used in the investigations. A three-electrode combination was employed which consisted of a saturated calomel electrode, a dropping mercury electrode and a platinum wire as the auxiliary electrode. A conventional H-type cell was maintained at 25 ± 1°C and all sweeps utilized a scan rate of 2mV/s and a drop time of 2s.

In Britton-Robinson buffer (pH 11.0) the instrumental parameters were: Applied potential range -1.0 to -2.5V; current 50μA full scale; height of mercury column 75cm; flow rate of mercury 1.265 mg/s; modulation amplitude, set at 50mV; and low pass filter, set at a time constant of 1s. The instrument was operated in the differential-pulse mode.

3.4.3 Analysis of Pharmaceutical Dosage Form of Zomepirac Sodium

Only one dosage form, 100 mg tablets, was available from the manufacturer.

Twenty tablets were weighed, finely powdered and an amount of powder corresponding to the weight of one tablet was accurately weighed into a 100ml beaker. Twenty ml of methanol were added and the sample stirred magnetically for 15 min. The mixture was transferred quantitatively into a 50ml volumetric flask, diluted to volume with methanol and then filtered through Whatman No.1 paper discarding the first 5ml of the filtrate. One ml of the filtrate was

transferred to the polarographic cell and 19ml of Britton-Robinson buffer, pH 11.0, were added. As previously described, the solution was purged for 10 min with nitrogen prior to recording the polarogram.

The amount of zomepirac sodium in the form of the free acid was computed by the direct comparison method, using a reference standard solution of zomepirac sodium (0.7649×10^{-3} M/l).

3.4.3.1 1) Content Uniformity Test of Zomepirac Sodium

Ten tablets were randomly selected from the sample. Each tablet was placed in an individual 150ml beaker, 20ml of methanol were added and the system was allowed to stand for 5 min in order to promote disintegration of the tablets. The remaining larger lumps of tablet mass were crushed with a glass rod and the mixture stirred magnetically for 20 min. After transferring the mixture quantitatively to a 50ml volummetric flask, the determination was continued as described in the previous section.

3.4.4 Macroscale Electrolysis of Zomepirac Sodium

The procedure used was similar to that for the controlled potential coulometry except the cell contained 200mg of zomepirac sodium in 25ml of 20% v/v methanol in 1N NaCl. The pH was adjusted to 11.0 with NaOH solution. The applied potential remained at - 1.8V while the reduction time was 8 hr. The initial yellow colour of the solution turned colourless upon completion of the reduction and then

the product together with the supporting electrolyte was separated from the mercury and freeze-dried.

3.4.5 Controlled-Potential Coulometry of Zomapirac Sodium

A PAR, Model 173, potentiostat-galvanostat, equipped with a PAR, Model 377A, three electrode coulometric cell system was connected to a Hi-Tek digital integrator and digital voltmeter.

Nineteen ml of Britton-Robinson buffer, pH 11.0, were placed in the coulometric cell on top of a 5ml layer of triple distilled mercury and 1ml of 10^{-3} M solution of zomepirac sodium in methanol was added. The system was purged for 10 min with purified nitrogen. The applied potential was set at - 1.8V with a current range of 10μA full scale and the solution was electrolyzed until the digital readout indicated a constant but small count. One hour was required to complete the electrolysis. The process was repeated with a blank consisting of 19ml of Britton-Robinson buffer and 1ml of methanol.

3.4.6 Controlled-Potential Coulometry of Deschlorozomepirac

Apparatus and conditions used were similar to those outlined for zomepirac sodium.

The experiment, however, was performed on a solution containing nineteen ml Britton-Robinson buffer pH 11.0, and 1ml of 1mg/ml methanolic solution of deschlorozomepirac on top of a 5ml layer of triple distilled mercury.

3.4.7 Cyclic Voltammetry of Zomepirac Sodium and Deschlorozomepirac

Cyclic voltammetric experiments at a hanging mercury drop electrode were performed with a four - component system consisting of a PAR EG & G, Model 175, Universal Programmer, a PAR, Model 173 potentiostat-galvanostat, a Houston Model 2000 Omnigraphic recorder and a PAR, Model 9323, hanging mercury drop electrode fitted with a polarographic cell.

Two supporting electrolyte systems were employed. In Britton Robinson buffer, pH 11.0, the parameters were : potential range, -1.1 to -1.65V; current range, 10μA; scan rate varied from 10 mV/s to 200mV/s.

In a dimethylformamide/lithium perchlorate system, the settings were : potential range, -0.4 to -2.2V; current range and scan rates were the same as in the previous system. 20ml of 5 x 10^{-4} M zomepirac sodium solution consisting of nineteen ml of Britton-Robinson buffer pH 11.0, and 1ml of 10^{-3} M methanolic solution of zomepirac sodium were used. For the experiment for deschlorozomepirac, a 20ml solution containing nineteen ml of Britton-Robinson buffer, pH 11.0, and one ml of 1mg/ml methanolic solution of deschlorozomepirac was employed in both instances, a 10 min purge with nitrogen was performed. Data for determining the effect of changing scan rate on the cyclic voltammetric peak currents of zomepirac sodium and deschlorozomepirac are presented in Tables 2 and 3 respectively.

3.4.8 Preparation of Calibration Graph

A stock solution of zomepirac sodium (10^{-3} M) was prepared in anhydrous methanol. Five test solutions of varying concentrations, 1 to 5 x 10^{-4} M were prepared by appropriately diluting the stock solution with Britton-Robinson buffer, pH 11.0. In the total sample volume of exactly 20ml, the amount of methanol was always maintained at 1ml.

All samples were purged with oxygen-free nitrogen for 10 min prior to each run and a stream of nitrogen was allowed to flow gently over the surface of the solution during the electroreduction. Samples of each of five concentrations were run five times and resulted in a correlation coefficient for the graph of 0.9992. Data for plotting the calibration curve are presented in table 4, while Fig 12 illustrates the calibration graph.

3.4.9 Diffusion Dependence Studies

These studies were carried out at pH 11.0 on a 5 x 10^{-4} M solution of zomepirac sodium. The applied potential was from -1.0 to -2.5V and the height of the mercury column ranged from 60 to 80cm. The weight of mercury was also obtained at each of five heights over that range. Table 1 presents data for the diffusion studies on zomepirac sodium.

3.4.10 Macroscale Electrolysis of o-(p-chlorobenzoyl) benzoic acid

A PAR, Model 173, potentiostat-galvanostat equipped with a PAR, Model 377 A, three component coulometric cell system was connected to a Hi-Tek digital integrator and digital voltmeter.

The procedure used was similar to that for the controlled potential coulometry of ketoprofen except that the cell contained 200 mg of o-(p-chlorobenzoyl) benzoic acid in 25ml of 20% v/v methanol in 1N NaCl. The pH was adjusted to 11.0 with NaOH solution. The applied potential was set at -1.8V while the reduction time was 6 hrs. Upon completion of the reduction, the product, together with the supporting electrolyte, was separated from the mercury, the mixture was acidified with 0.2N H_2SO_4 and then extracted with ether. The ethereal solution was washed with distilled water and dried over magnesium sulphate. It was concentrated to about 2ml and left to stand overnight to give white needle-like crystals in about 60% yield. The product melted between 122-123°C. (NMR 100 MHz, CD_3OD) δ 6.6 (s,1H), 7.4 (m,4H) 7.7 (m,2H) 7.95 (m 2H). (see discussion) M^+ m/e 244, base peak m/e 209. IR (KBr disc: 3000 /cm, 1770/cm , 1600/cm , 1300/cm , 1220/cm , 1090/cm , 1000/cm. Elemental analysis C, 67.8%, H, 3.69% ; 0, 13.03%; Cl; 15.07%. The spectra of the foregoing data are presented in Figures 14 to 19.

3.4.11 Differential-Pulse Polarographic Analysis of Ketoprofen

The apparatus and conditions for the analysis of ketoprofen were similar to those used for the analysis of zomepirac sodium except that the pH of the Britton-Robinson buffer was 6.0 and the flow rate of mercury was 1.26mg/sec.

3.4.12 Controlled-Potential Coulometry of Ketoprofen

A PAR, Model 173, Potentiostat-Galvanostat, equipped with a PAR, Model 377A, three component coulometric cell system was connected to a Hi-Tek digital integrator and digital voltmeter.

Nineteen ml of Britton-Robinson buffer, pH 6.0, were placed in the coulometric cell on top of a 5ml layer of triple distilled mercury and 1ml of 10^{-3} M solution of ketoprofen in methanol was added. The system was purged for 10 min with purified nitrogen, the applied potential was set at -1.25V with a current range of 1mA full scale and the solution was electrolysed until the digital readout indicated a constant but small current count. The electrolysis time was 40 min. The process was repeated with a blank consisting of 19 ml of Britton-Robinson buffer and 1ml of methanol.

3.4.13 Cyclic Voltammetry of Ketoprofen

Cyclic voltammetric experiments at a hanging mercury drop electrode were performed with a four-component system

consisting of a PAR EG & G, Model 175, Universal Programmer, a PAR, Model 173, Potentiostat-Galvanostat, a Houston Model 2000 Omnigraphic recorder and a PAR, Model 9323, hanging mercury drop electrode fitted with a polarographic cell.

Two supporting electrolyte systems were employed. In Britton Robinson buffer, pH 6.0, the parameters were : potential range, -0.8 to -1.4V; current range, 10μA; scan rate varied from 10mV/s to 200mV/s. In a dimethylformamide/tetraethylammonium bromide system, the settings were : potential range, -0.5 to -2.2V; current range and scan rates were the same as in the previous system. Data obtained from this experiment are presented in Table 7.

3.4.13.1 Preparation of Calibration Graphs

A stock solution of ketoprofen (10^{-3} M) was prepared in anhydrous methanol. Five test solutions of varying concentrations, 1 to 5 x 10^{-4} M, were prepared by appropriately diluting the stock solution with Britton-Robinson buffer, pH 6.0. In the total sample volume of exactly 20ml, the amount of methanol was always maintained at 1ml.

All samples were purged with purified nitrogen for 10 min prior to each scan and a stream of nitrogen was allowed to flow gently over the surface of the solution during the electroreduction. Samples of each of five concentrations were run five times and resulted in a correlation coefficient for the graph of 0.9994. Figure 13 presents the

calibration graph for ketoprofen while data for ploting the graph are presented in Table 5.

3.4.14 Diffusion Dependence Studies

These studies were carried out at pH 6.0 on a 5×10^{-4} M solution of ketoprofen. The applied potential was set at -1.15V and the height of the mercury column ranged from 60 to 80cm. The mercury flow rate was also obtained at each of five heights over that range. Table 6 presents the data obtained for this experiment.

3.4.15 Analysis of Pharmaceutical Dosage Forms of Ketoprofen

Two dosage forms, 50mg capsules and 100mg suppositories, were available from the manufacturer.

3.4.15.1 Analysis of Capsules

The contents of twenty capsules were transferred quantitatively into a tared 100ml beaker and weighed. The powder was throughly mixed and an amount of powder corresponding to the weight of one capsule content was accurately weighed into a 100ml beaker. Twenty ml of methanol were added and the sample stirred magnetically for 15 min. The mixture was transferred quantitatively into a 50ml volumetric flask, diluted to volume with methanol and then filtered through a Whatman No. 1 paper discarding the first 5ml of the filtrate. One ml of the filtrate was transferred to the polarographic cell and 19ml of Britton-Robinson buffer, pH 6.0, were added and the solution

was purged for 10 min with nitrogen prior to recording the polarogram. The amount of ketoprofen was computed by the direct comparison method, using a reference standard solution of ketoprofen (0.3937×10^{-3} M). Experimental data are presented in Table 10.

3.4.15.2 Content Uniformity Test

Ten capsules were randomly selected from the sample. The content of each capsule was quantitatively transferred into an individual 100ml beaker, 20ml of methanol were added and then stirred magnetically for 20 min. After transferring the mixture quantitatively to a 50ml volumetric flask, the assay was continued as described in the previous section. (See Table 11 for the experimental data)

3.4.15.3 Analysis of Suppositories

Ten suppositories were randomly selected and weighed. Each was placed in an individual 150ml beaker, 50ml of methanol were added and then warmed until complete dissolution was achieved. The mixture was stirred magnetically for 10 min while still warm and then cooled in a refrigerator for 40 min. The resulting agglomerate was allowed to stand for 10 min at room temperature and following this, it was stirred with a glass rod to form a slurry, transferred quantitatively to a 100 ml volumetric flask and diluted to volume with methanol. After filtering through a Whatman No 1 paper, a 1ml aliquot of the solution was treated in the manner described under the analysis of

capsules. Data obtained for this experiment are presented in Table 12.

3.4.16 Macroscale Electrolysis and Isolation of the Reduced Product of Ketoprofen

The procedure for electrolysis was similar to that for the controlled potential coulometry except that the cell contained 180mg of ketoprofen in 50ml of methanol to which was added 20ml of 1N aqueous NaCl as supporting electrolyte. The pH was adjusted to 6.0 by the dropwise addition of 0.2N HCl. The applied potential was set at -1.25V and the reduction time required 4 hrs. The solution was acidified with 0.1N H_2SO_4 and then extracted with chloroform. The chloroform layer was washed with distilled water and dried over magnesium sulphate (anhydrous). The final solution which was concentrated to about 0.5ml was cooled in a refrigerator overnight and yielded 97mg of a white crystalline powder that melted over the range 113-115°C.The following physical-chemical parameters were obtained. UV (MeOH), λ max, 212nm, logε= 4.37; 252nm, logε= 4.03 : IR (KBr), 3420/cm, 3000/cm, 2700/cm, 1720/cm, 1610/cm, 1460/cm, 1240/cm, 1020/cm, 900/cm: NMR (60 MHz, DMSO-d_6), δ 7.3 (m,9H), 5.6 (m,2H), 3.6 (q,1H), and1.3 (d,3H) (see discussion). The spectra for the foregoing data are presented in Figures 35-39.

Results and Discussion

3.5

Only results and brief discussions will be presented here since effects of the various parameters that affect polarographic behaviour of electroactive species have already been discussed under minoxidil.

3.5.1 Temperature Control for Polarographic Experiments

The temperature of the cell was maintained at a constant value of 24 ± 1°C, since at this temperature well developed waves were obtained.

3.5.2 Determination of Optimum Height of Mercury Column

Comparison of polarograms obtained at different heights of mercury column showed no significant differences. The height of the mercury column was varied over the range from 60 to 80cm, with a 5cm interval between settings. Although, changes in the height of mercury column affect the number of mercury drops per unit time. Such an effect was not apparent in this work since an automatic drop timer was used to control the dropping time. Well developed d.c. and d.p. polarographic waves were exhibited at each of the heights investigated. A mercury column with a height of 75 cm was chosen because it yielded well-resolved, easily measured peaks and resulted in flow rates of 1.265 and 1.26mg/sec for zomepirac sodium and ketoprofen respectively at a dropping time of 2sec.

3.5.3 Determination of Optimum Buffer or Supporting Electrolyte System

Britton-Robinson buffer was chosen for the investigation because of its versatility and wide pH range. The buffers (pH range 6.0 to 11.0) were screened polarographically and were found not to contain any substance that might interfere with the reduction of zomepirac sodium or ketoprofen. Buffers with pH values below 6.0 were not used because they cause the precipitation of zomepirac and ketoprofen free acids from solution.

Both compounds exhibit well-developed d.c and d.p polarographic waves in the pH range 6.0 to 11.0. At a pH of 11.0, however, zomepirac exhibits one well-developed and sharply defined peak that is suitable for analytical application. Britton-Robinson buffer pH 11.0 was, therefore, chosen as the supporting electrolyte system for the analysis of zomepirac sodium. The most suitable peak for ketoprofen, however, was observed at a pH of 6.0.

3.5.4 Determination of Solution Stability

Methanolic solutions of zomepirac sodium and ketoprofen have been found to be stable for at least five days. Comparison of polarograms obtained for freshly prepared solutions to those for five day old solutions show no significant differences.

3.5.5 Determination of pH Change Before and After Electrolysis

The pH values of the buffered zomepirec sodium and ketoprofen solutions showed no significant differences after electrolysis. Consequently, the pH at which the electrochemical reduction occurred was recorded as being identical to that prior to the reduction.

3.5.6 Determination of Diffusion Dependency

In order to investigate the nature of the limiting current observed for the reduction of zomepirac sodium and ketoprofen in Britton-Robinson buffer pH 11.0 and 6.0 respectively, plots of the diffusion currents versus the square root of the corrected heights of mercury column were made as illustrated in Figures 3 and 4.Data for preparing these plots are presented in Tables 1 and 6. Both plots resulted in straight lines that did not pass through the origin. The graph for zomepirac sodium has a slope of $0.228\mu A/cm^{1/2}$, while that for ketoprofen is $0.285\mu A/cm^{1/2}$. Although, the electrochemical reduction process might be regarded as being essentialy diffusion controlled, other parameters play some role in the actual reduction mechanism.

3.5.7 Controlled Potential Coulometry

The controlled potential coulometric experiments on zomepirac sodium, deschlorozomepirac and ketoprofen reveal that two electrons per molecule of each species were

Table : 1

Data for Diffusion Dependence Studies for Zomepirac Sodium in Britton-Robinson Buffer, pH 11.0

Height of Hg column/cm	Peak height (μA) 1	2	3	Average i_p	Hg Flow Rate m	Drop time t (sec)	Back pressure $h = 3.1/m^{\frac{1}{3}} t^{\frac{1}{3}}$	Corrected Hg Height h	$h^{\frac{1}{2}}$
80	1.68	1.72	1.76	1.72	1.30	2.0	2.26	77.75	8.82
75	1.65	1.75	1.70	1.70	1.27	2.0	2.28	72.73	8.53
70	1.70	1.55	1.56	1.60	1.13	2.0	2.36	67.64	8.22
65	1.64	1.53	1.55	1.57	1.03	2.0	2.44	62.56	7.91
60	1.53	1.37	1.45	1.46	0.983	2.0	2.48	57.53	7.58

m = mg/sec

FIGURE 3: Graph of Diffusion Current of Zomepirac Sodium in Britton- Robinson Buffer, pH 11.0 vs. Square Root of Corrected Height of Mercury Column.

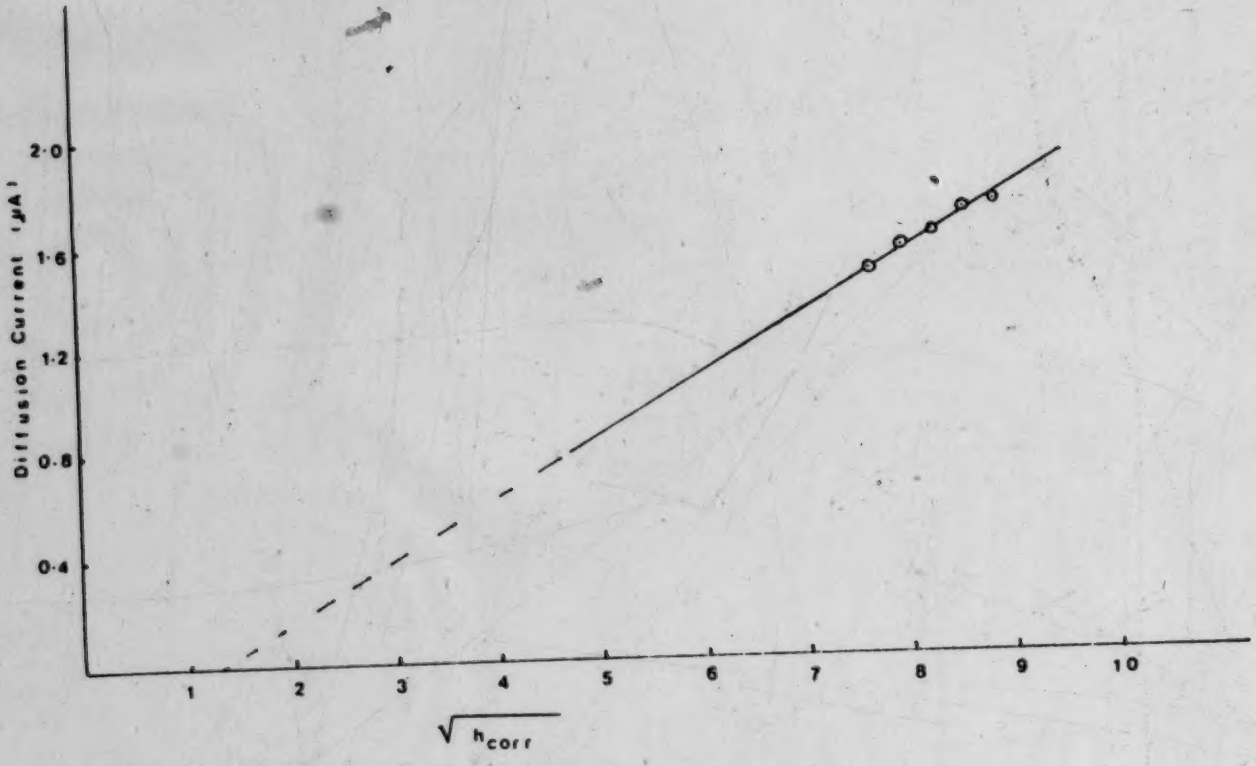
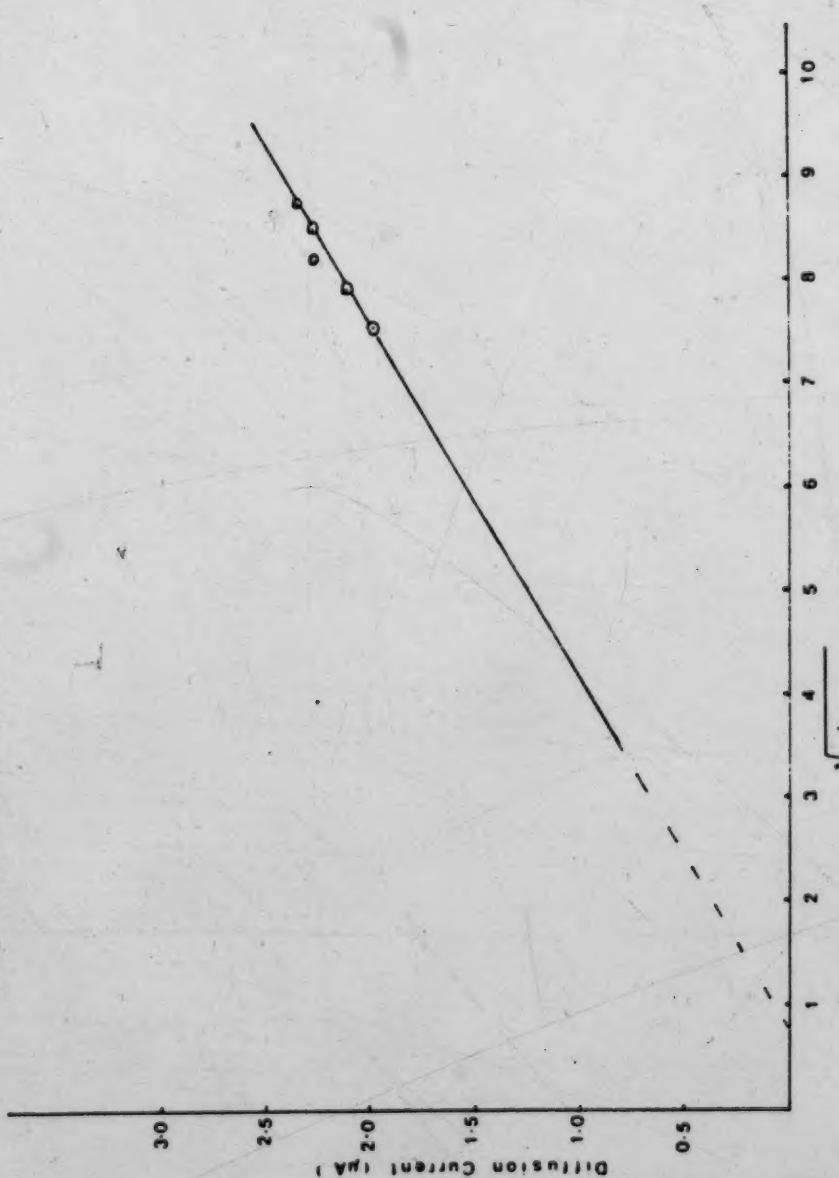


FIGURE 4: Graph of Diffusion Current of Ketoprofen in Britton-Robinson Buffer, pH 6.0 vs Square Root of Corrected Height of Mercury Column



involved in the electrochemical reduction process in each instance. The reduction process might involve only one bond in each of the species investigated, since only two electrons are involved in a complete cleavage or formation of a chemical bond. Among the functional groups which are possible sites of reduction in these molecules, the carbonyl carbon-oxygen double bond is the most readily reducible. The realization that two electrons per molecule were involved in the reduction process indicates that only one of the C = O bonds has undergo reduction. The observed $E_{\frac{1}{2}}$ values and the shapes of the polarographic waves strengthens the postulation that the site of the electrochemical reduction is the carbonyl group.

3.5.8 Cyclic Voltammetry of Zomepirac Sodium

Only one reduction peak was observed in the cyclic voltammetric experiment in Britton-Robinson buffer pH 11.0 (Figure 5) where the E occurs at -1.53V. No reverse anodic peak was observed under the sweep rate range studied. Detailed analysis of the cathodic peak indicated a peak potential shift of 40 to 50mV as the sweep rate was varied from 200mV/s to 10mV/s. The peak current varied linearly with the square root of scan rate over this range indicating the absence of any complicated homogenous reaction on the time scale of the experiment (Figure 6). A plot of $i_p / \nu^{\frac{1}{2}}$ <u>versus</u> ν is independent of scan rate implying a predominantly diffusion controlled process (see Figure 7). See Table 2 for

Table : 2

Data for Determining the Effect of Scan Rate on the Cyclic Voltammetric Peak current of Zomepirac Sodium in Britton-Robinson Buffer, pH 11.0.

Current i_p (μA)	Scan rate ν mVs^{-1}	$\nu^{1/2}$	$ip/\nu^{1/2}$
3.14	200	14.14	0.222
2.30	100	10.00	0.230
1.65	50	7.07	0.233
1.00	20	4.47	0.223
0.80	10	3.16	0.253

Table : 3

Data for Determining the Effect of Scan Rate on the Cyclic Voltammetric Peak Current of Deschlorozomepirac in Britton-Robinson Buffer, pH 11.0.

Current i_p (μA)	Scan rate ν mV/s	$\nu^{1/2}$	$i_p/\nu^{1/2}$
2.43	200	14.14	0.17
1.74	100	10.00	0.172
1.36	50	7.07	0.192
1.36	20	4.47	0.19
0.64	10	3.16	0.20

FIGURE 5: Cyclic Voltammogram of Zomepirac Sodium in Britton-Robinson Buffer, pH 11.0

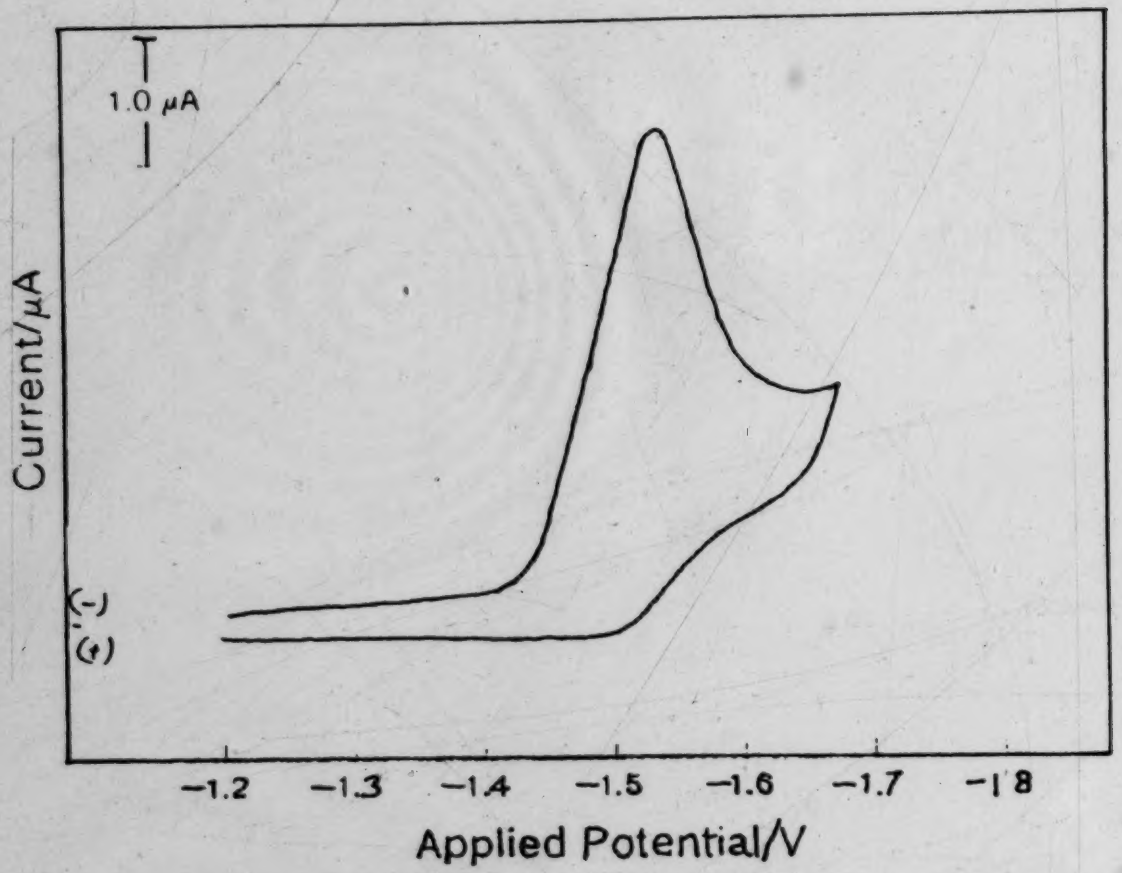


FIGURE 6: Graph of Cyclic Voltammetric Peak Current for Zomepirac Sodium *vs.* the Square Root of Scan Rate.

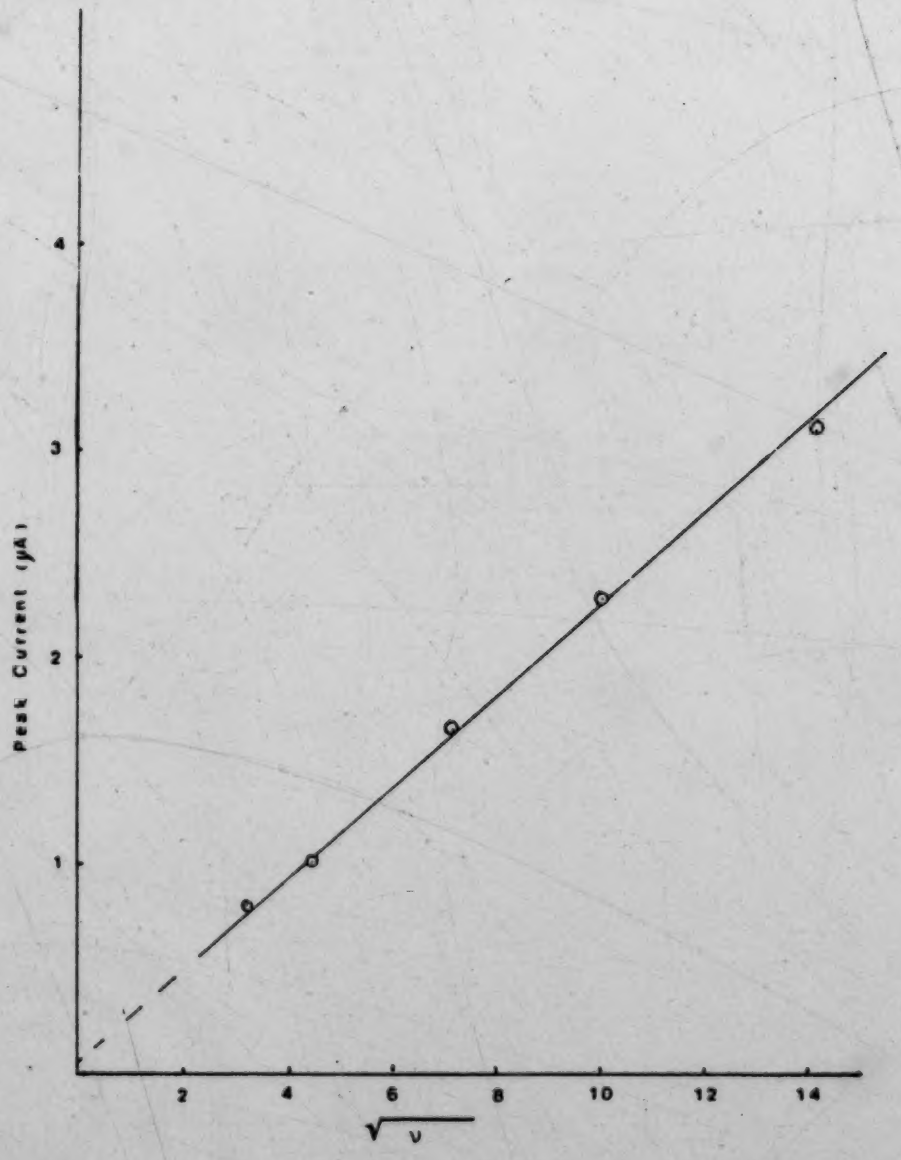


FIGURE 7: A Plot of $i_p/\nu^{1/2}$ vs ν for Zomepirac Sodium in Britton-Robinson Buffer, pH 11.0

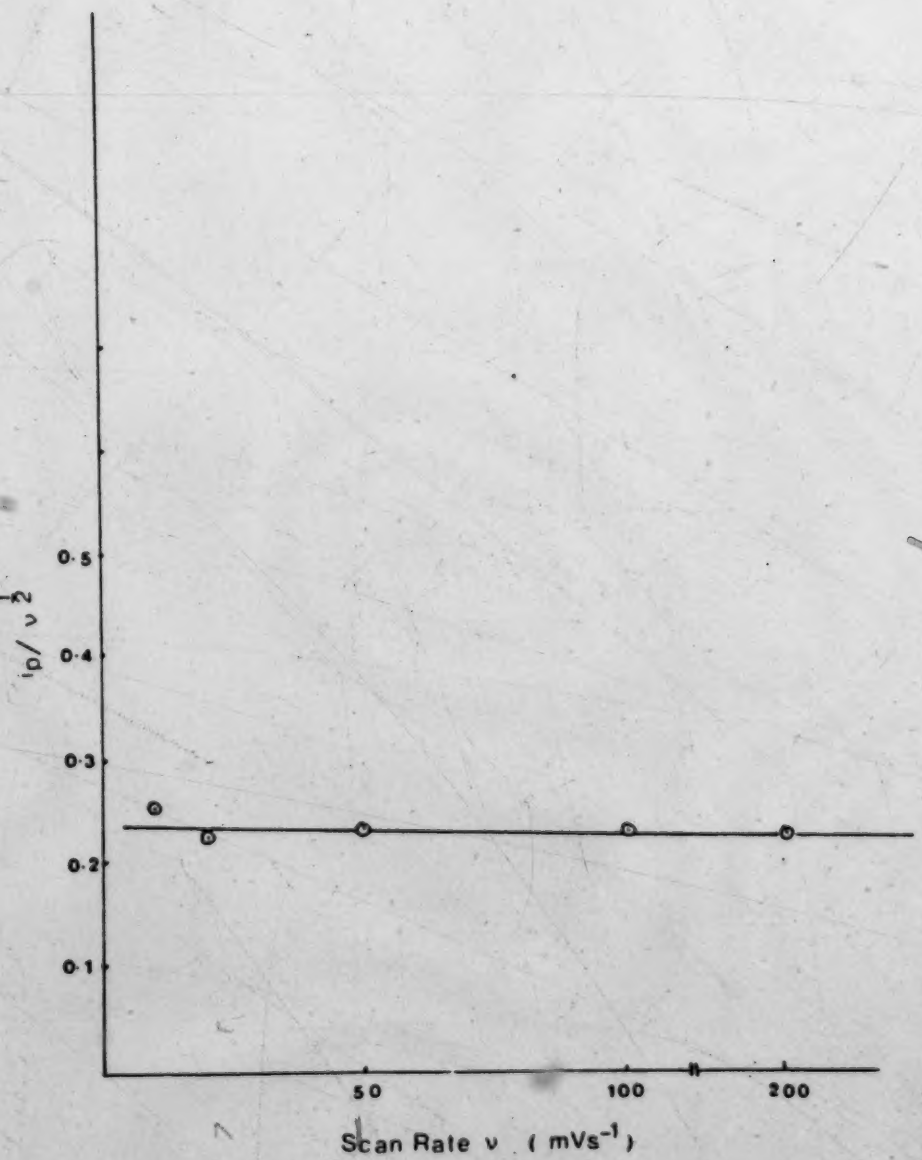
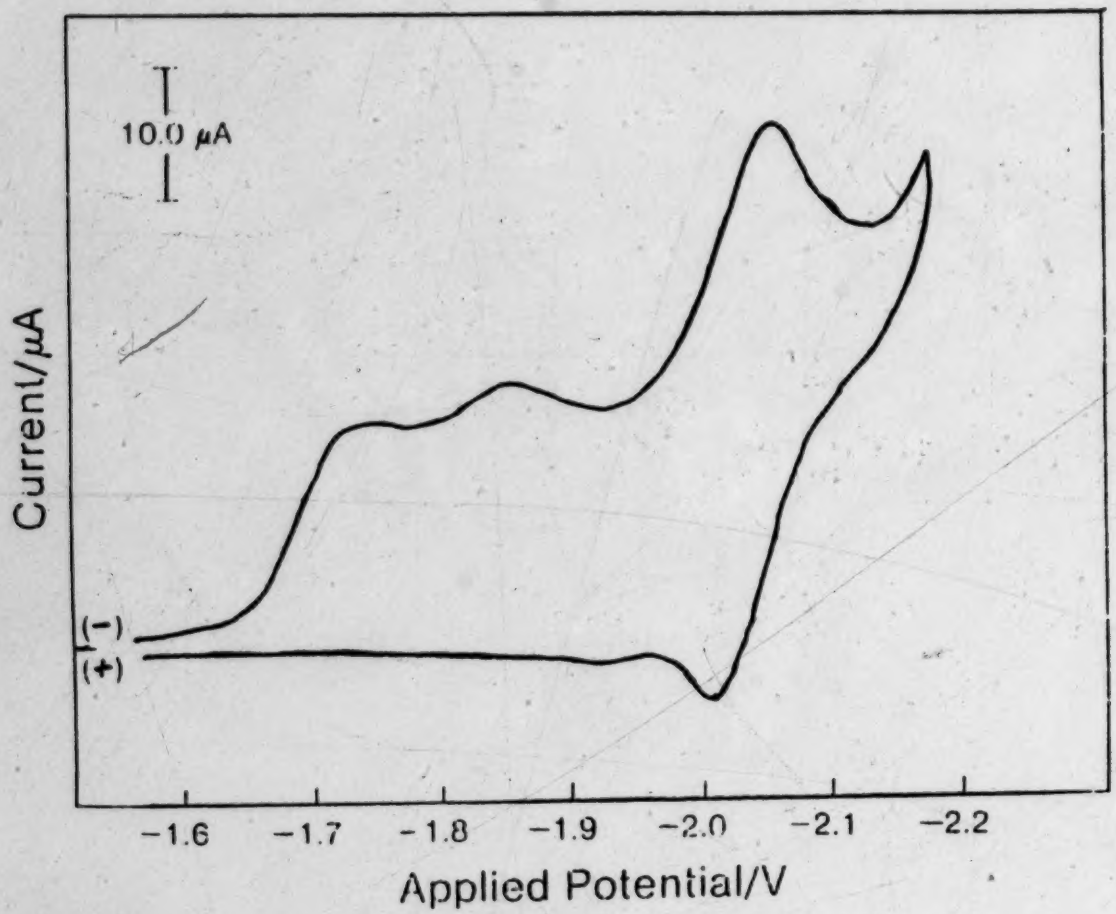


FIGURE 8: Cyclic Voltammogram of Zomepirac Sodium in DMF/LiClO.



the experimental data. The cyclic voltammogram of zomepirac sodium in DMF/$LiClO_4$ shows three cathodic peaks and one reverse anodic peak. The E_{pc} values occured at -1.75V, -1.85V and -2.08V. The height of the peak at -2.08V was twice that of either of the first two peaks. The third peak may be the result of a two electron process while the first two peaks each involved a one electron process. The E_{pa} was at -1.98V (see Figure 8).

3.5.9 Cyclic Voltammetry of Deschlorozomepirac

Deschlorozomepirac exhibits only one cyclic voltammetric cathodic peak in Britton-Robinson buffer pH 11.0 (Figure 9). The E_{pc} value was -1.55V. No reverse anodic peak was observed under the sweep rate range studied. The cathodic peak potential shifts anodically by about 30mV as the sweep rate was varied from 200mV/s to 10mV/s. The peak current varied linearly with the square root of scan rate over this range. A plot of $i_p/\nu^{1/2}$ <u>versus</u> ν is independent of scan rate as shown in Figure 10. The experimental data is presented in Table 3. In DMF/$LiClO_4$ system, however, deschlorozomepirac exhibits two distinct cathodic peaks, with E values at -1.65V and -1.83V. Two reverse anodic peaks can also be distinguished which have E_{pa} values at -1.76 and -1.95V (Figure 11).

The shape of the cylic voltammogram of deschlorozomepirac is similar to that of zomepirac sodium except that in the non-aqueous system, three cathodic waves

FIGURE 9: Cyclic Voltammogram of Deschlorozomepirac in Britton-Robinson Buffer, pH 11.0

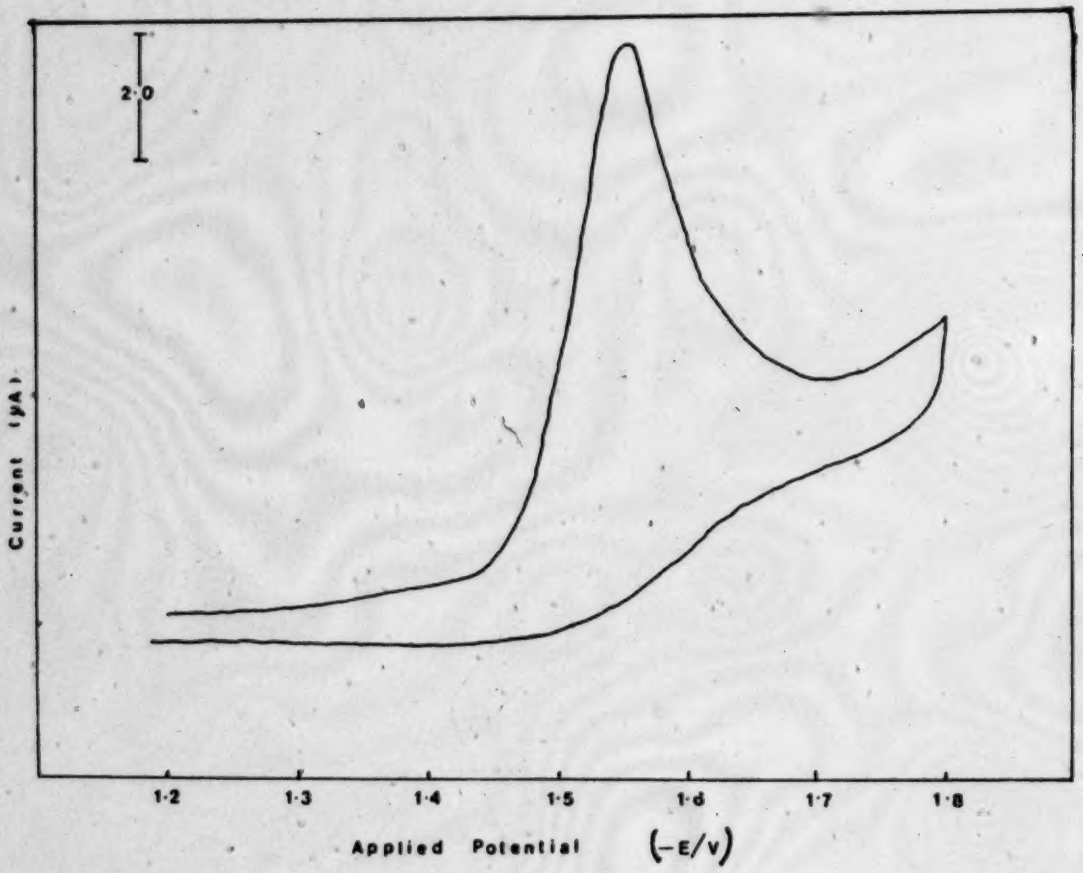


FIGURE 10: Graph of $i_p/\nu^{1/2}$ vs ν for Deschlorozomepirac in Britton-Robinson Buffer, pH 11.0.

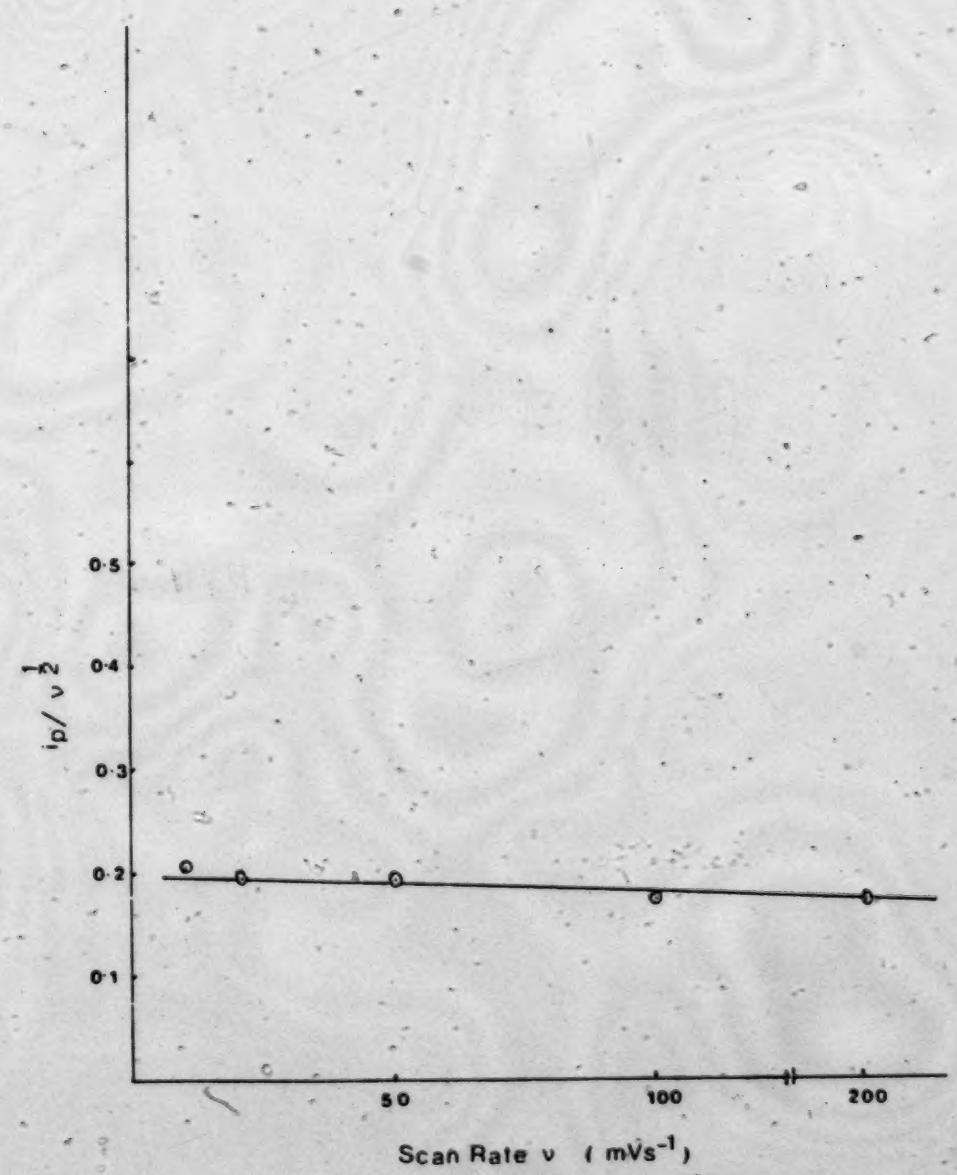
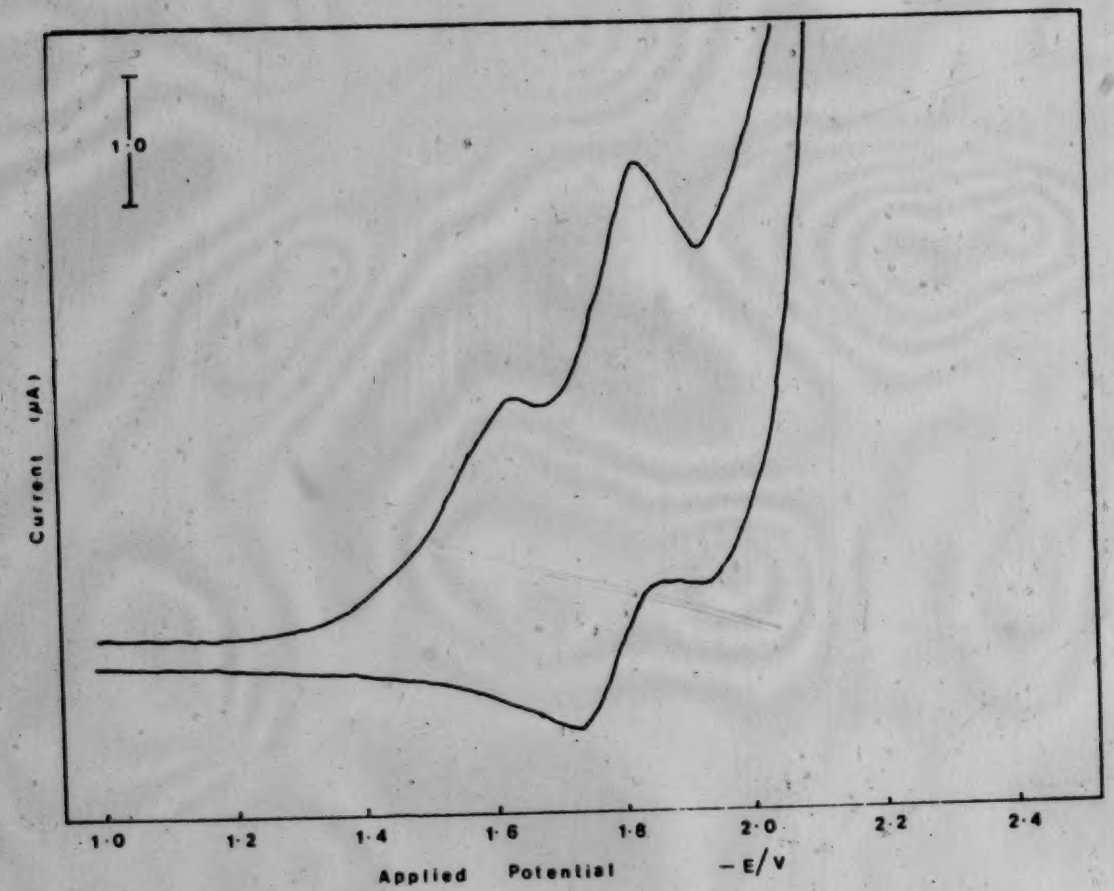


FIGURE 11: Cyclic Voltammogram of Deschlorozomepirac in DMF/LiClO.



could be distinguished for zomepirac sodium while only two waves are observed for deschlorozomepirac. In the non-aqueous system, therefore, another electrochemical process in addition to the reduction of the carbonyl group probably takes place for zomepirac. It is probable that this additional electrochemical process might be a one electron reduction of the carbon-chlorine bond.

3.5.10 Preparation of Calibration Graphs

Calibration graphs for zomepirac sodium and keteprofen were pepared as described in the experimental section. Both compounds exihibit linear relationships between their d.p. polarographic peak currents and the concentration of compound, within the concentration range $1x10^{-4}$M to $5x10^{-4}$M. Excellent correlation coefficients were obtained for each compound. The value for ketoprofen is 0.9994 while that for zomepirac sodium is 0.9992. Experimental data are presented in Tables 4 and 5 respectively while Figures 12 and 13 illustrate the corresponding calibrations graphs. On extrapolation, both graphs shows slight deviations from the origin.

3.5.11 Macroscale Electrolysis of *o*-(*p*-chlorobenzoyl) benzoic acid

Figures 14 and 15 show the NMR spectra of *o*-(*p*-chlorobenzoyl) benzoic acid and that of the electrochemically reduced product respectively. An

Table : 4

Data for Calibration Graph for Zomepirac Sodium in Britton -Robinson Buffer, pH 11.0.

Reference soln conc M x 10^{-4}	d.p.p Peak Height(μA) 1	2	3	4	5	Average peak height(μA)	Correlation Coefficient
1	1.75	1.68	1.75	1.75	1.70	1.72	
2	3.40	3.40	3.40	3.40	3.45	3.41	
3	5.40	5.42	5.41	5.35	5.35	5.38	0.9992
4	7.05	7.00	7.08	7.05	7.10	7.05	
5	8.40	8.45	8.40	8.70	8.80	8.55	

Table : 5

Data for Calibration Graph for Ketoprofen in Britton -Robinson Buffer, pH 6.0

Sample conc x 10^{-4}M	D.P.P. Peak Current (μA) 1	2	3	Average D.p.p. Peak current
1.0	6.70	6.67	6.8	6.75
2.0	13.50	13.50	13.51	13.50
3.0	19.75	20.00	20.25	20.00
4.0	26.25	26.00	26.60	26.25
5.0	31.75	31.80	31.71	31.75

1. Coefficient of correlation = 0.9994

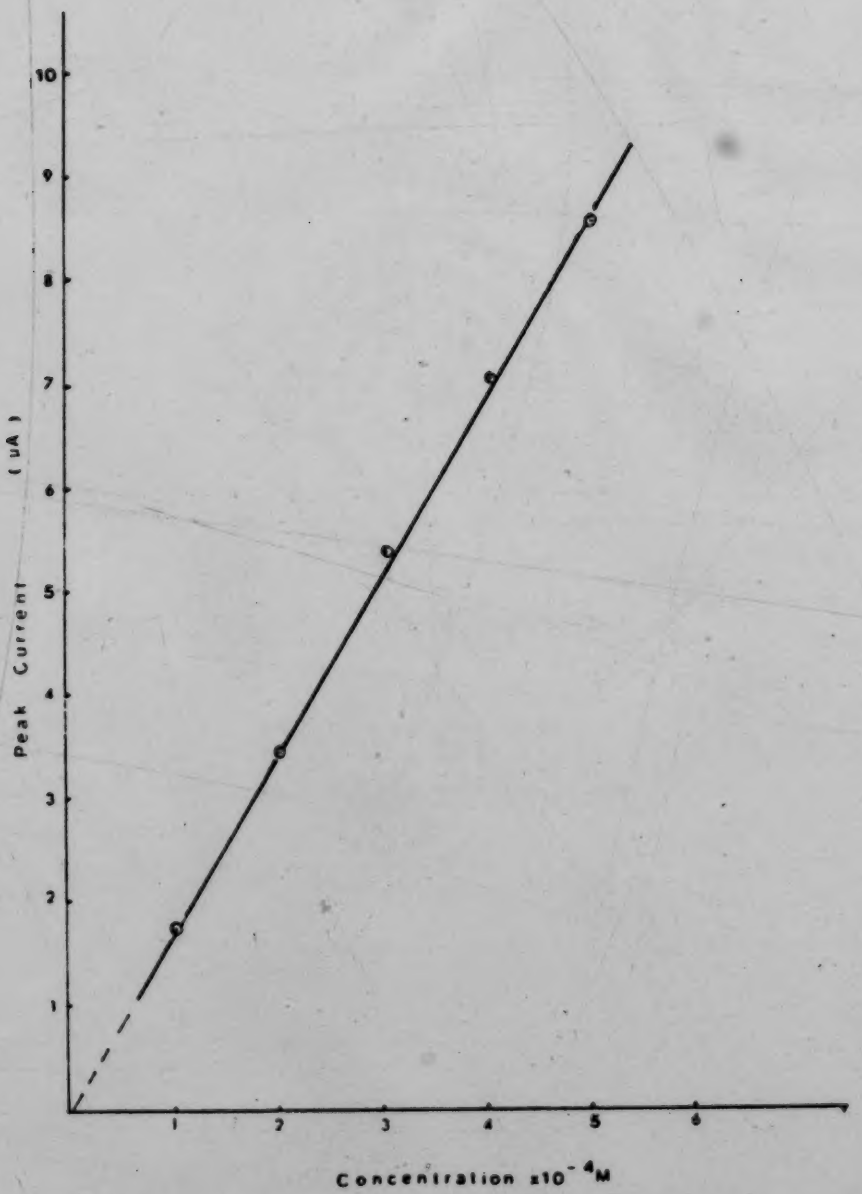


FIGURE 12: Calibration Graph for Zomepirac Sodium in Britton-Robinson Buffer, pH 11.0.

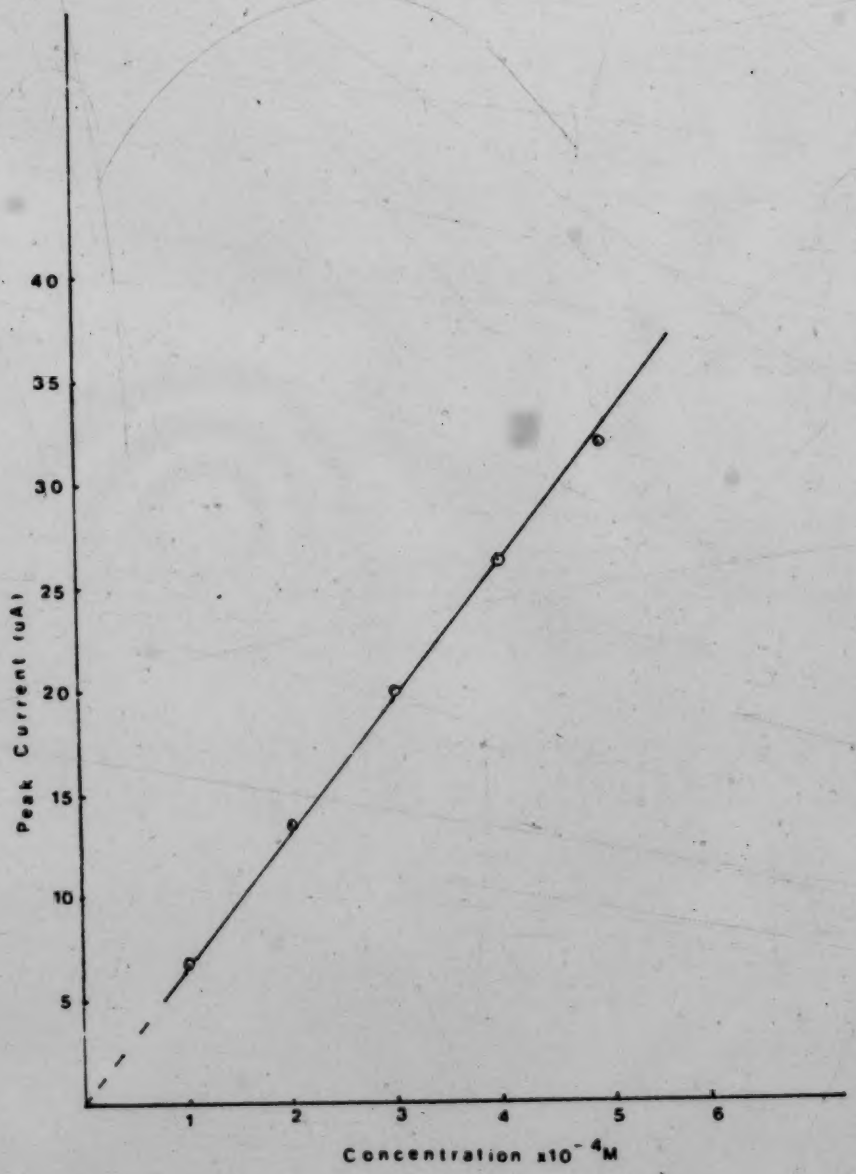


FIGURE 13: Calibration Graph for Ketoprofen in Britton-Robinson Buffer, pH 6.0

FIGURE 14: NMR Spectrum of *o*-(*p*-Chlorobenzoyl)benzoic Acid

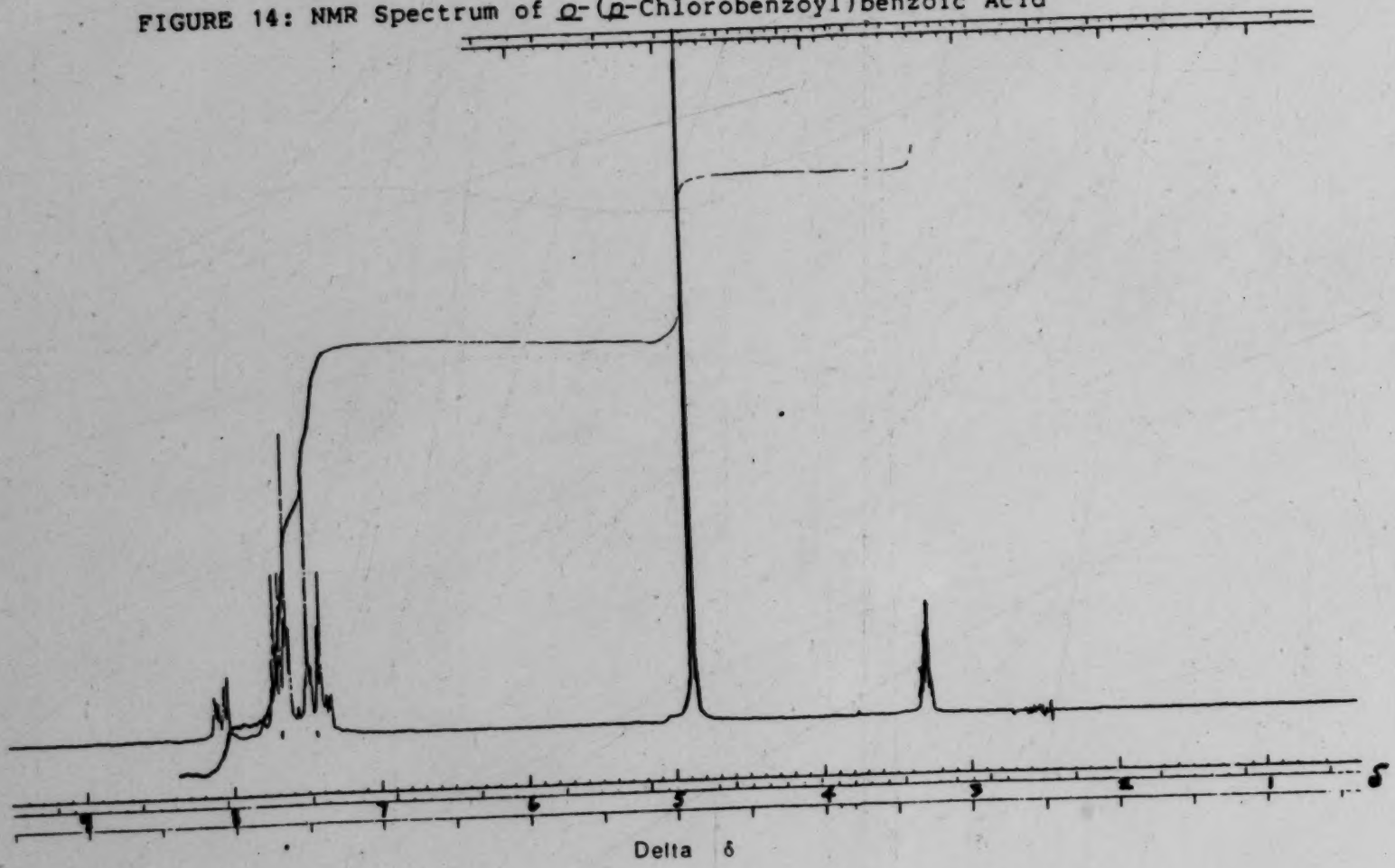


FIGURE 15: NMR Spectrum of Reduced *o*-(*p*-Chlorobenzoyl)benzoic Acid

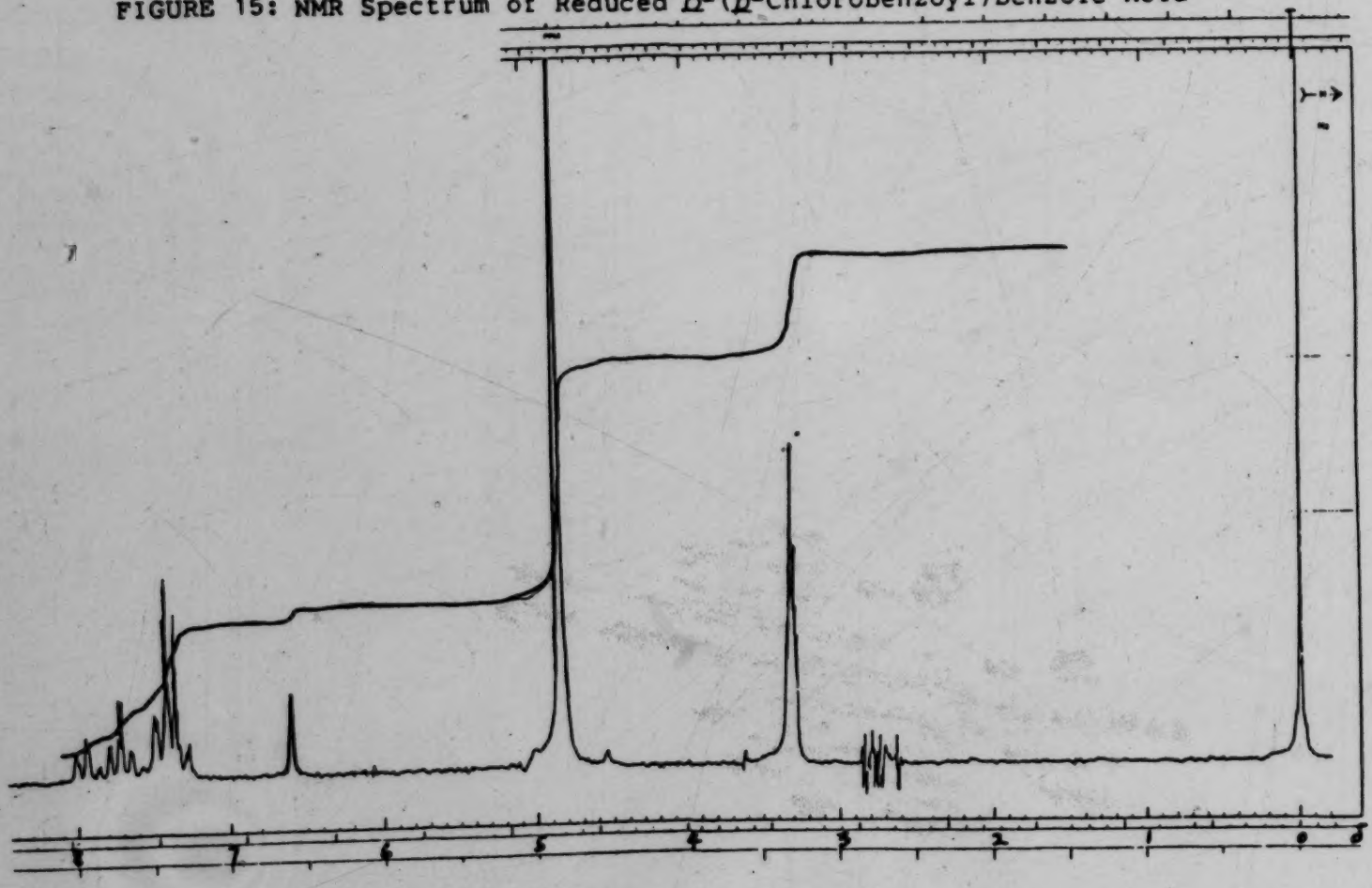
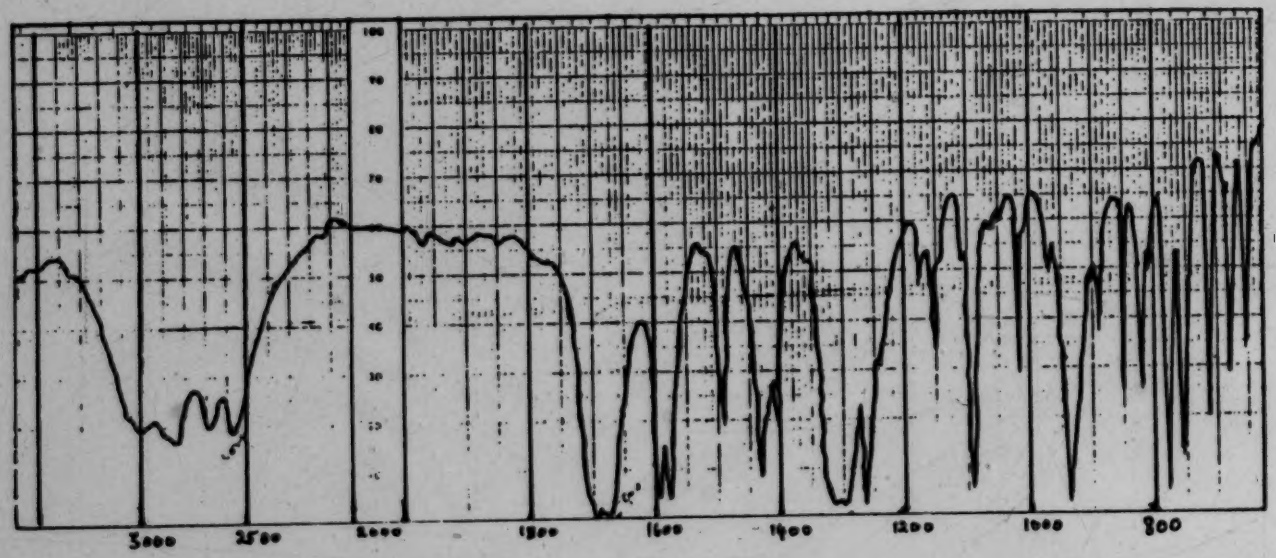


FIGURE 16: IR Spectrum of *o*-(*p*-Chlorobenzoyl)benzoic Acid



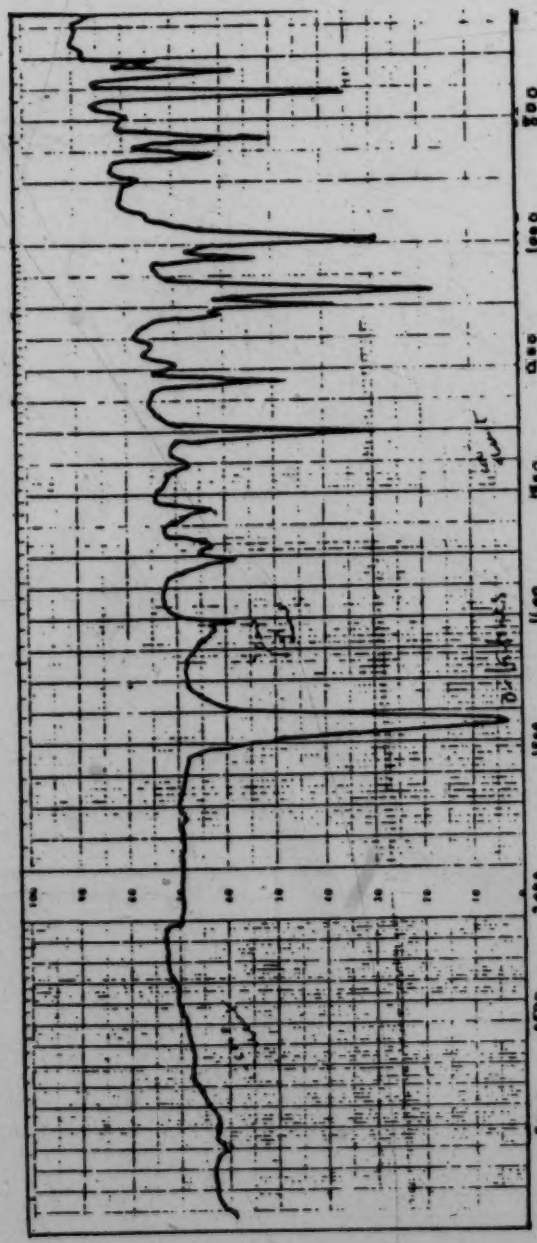


FIGURE 17: IR Spectrum of Reduced Ω-(Ω-Chlorobenzoyl)benzoic Acid

FIGURE 18: Mass Spectrum of Reduced o-(p-Chlorobenzoyl) benzoic Acid

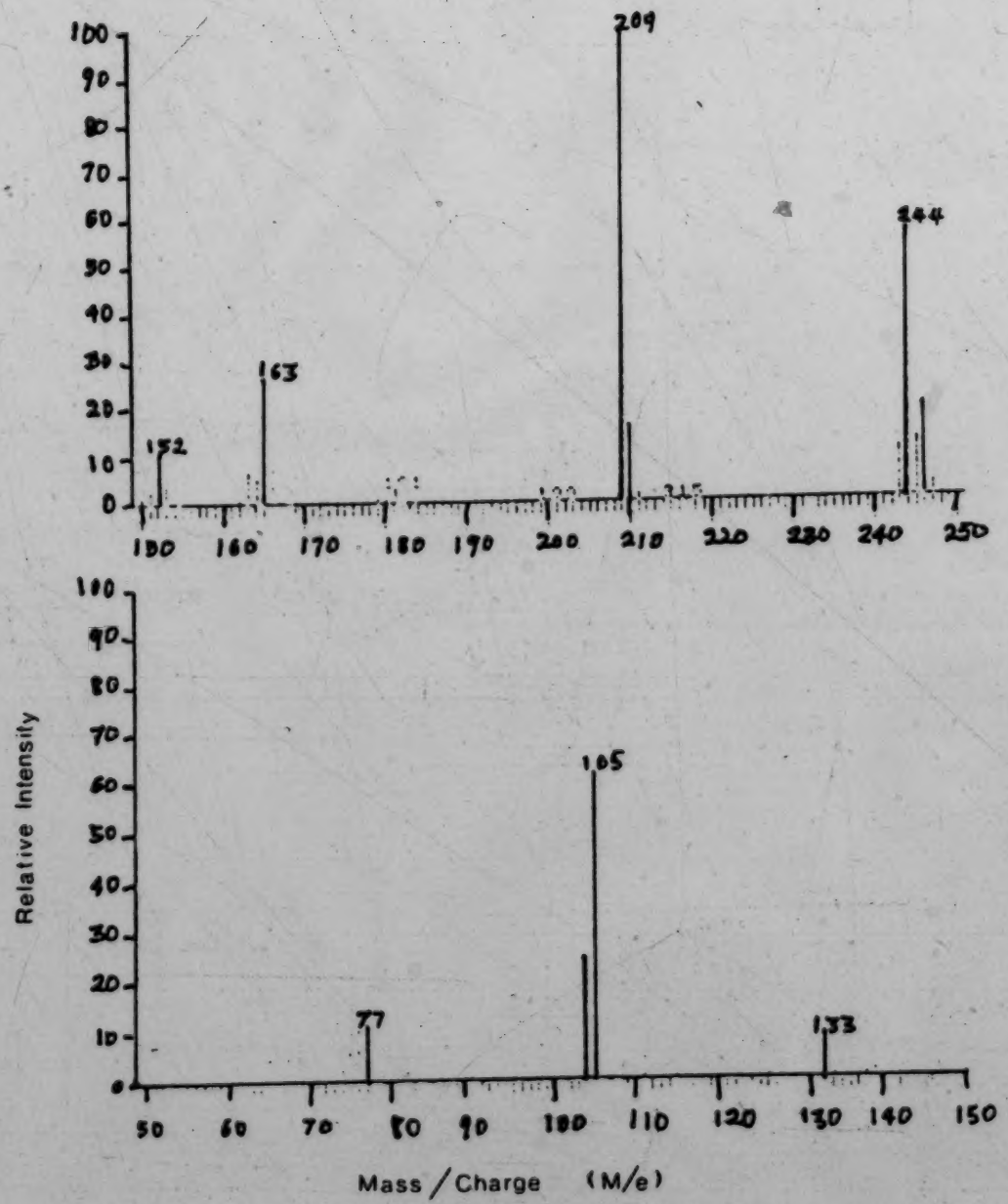
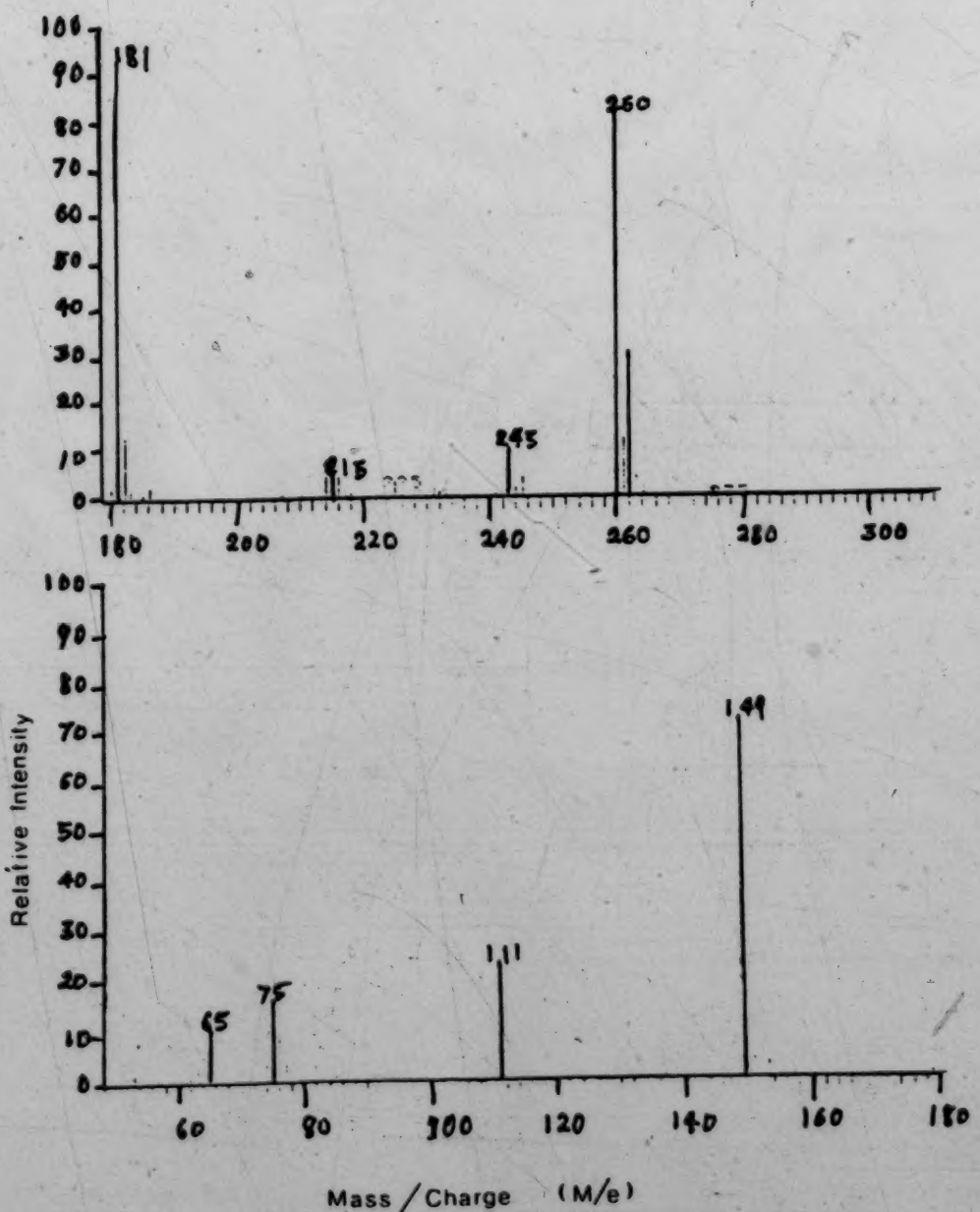


FIGURE 19: Mass Spectrum of *o*-(*p*-Chlorobenzoyl) benzoic Acid



additional peak with an integration value corresponding to a single proton and appearing at a delta value of 6.6 is observed in Figure 15. The rest of the spectrum is similar to that of the starting material. D_2O exchange has no effect on the spectrum of the reduced product implying that the additional proton is attached to a carbon atom. Figures 16 and 17 are the IR spectra of the starting material and the reduced product respectively. A strong peak is observed in Figure 16 at a wavenumber of 1770/cm. The carboxylic acid peaks (2500-3000/cm) were absent in this spectrum. There is also an absence of an O-H peak at about 3500/cm implying an absence of a hydroxy group in the structure of the reduced product.

Figure 18 shows the mass spectrum of the reduced product in which the molecular ion has a mass to charge ratio of 244.02. The mass spectrum of the starting material is shown in Figure 19 for comparative purposes. The reduced product has a molecular mass of sixteen atomic mass units less than that of the starting material. Probably, this could be due to a loss of an oxygen atom.

From the observations outlined above it can be proposed that the electrochemical reduction of this compound involves the benzoyl ketone to give a benzhydrolyl-derivative which then undergoes lactonization with the carboxylic acid portion of the molecule to give a γ-lactone derivative Figure 20. Data obtained from the elemental analysis of the

product agree excellently with the proposed product.

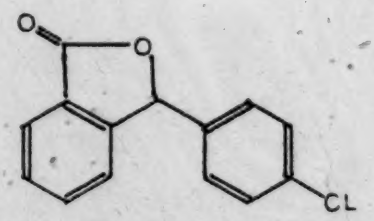


Figure 20.

3.5.12 Polargraphic Behaviour of Zomepirac Sodium

Zomepirac sodium exhibits three d.c and d.p polarographic waves in Britton-Robinson buffer over the pH range 6.0 to 11.0 (Figure 21 and 22). At pH 6.0, a single well-resolved wave is observed with an $E_{1/2}$ value of -1.28V. This wave is pH sensitive and the $E_{1/2}$ moves cathodically with increasing pH. The second wave appears at more negative potentials within the pH range 6.8 to 10.0 as illustrated by trace B in Figure 21. The $E_{1/2}$ value of this wave occurs at -1.45V and is independent of pH. At pH 11.0, the two waves shown in trace B merge to form one wave, C, with an $E_{1/2}$ value at -1.5V. This latter wave is well-resolved, intense and is suitable for quantitative work. The corresponding differential pulse waves are presented in Figure 22.

The electrochemical reduction of phenylketones has been extensively studied by various earlier workers (25-28). Elving *et al.* (25) have discussed the mechanism of the electrochemical reduction of phenylketones. They found that

FIGURE 21: Sampled D.C. Polarographic Waves for Zomepirac Sodium in Britton Robinson Buffer, pH Range A; 6.0, B; 8.0, C; 11.0.

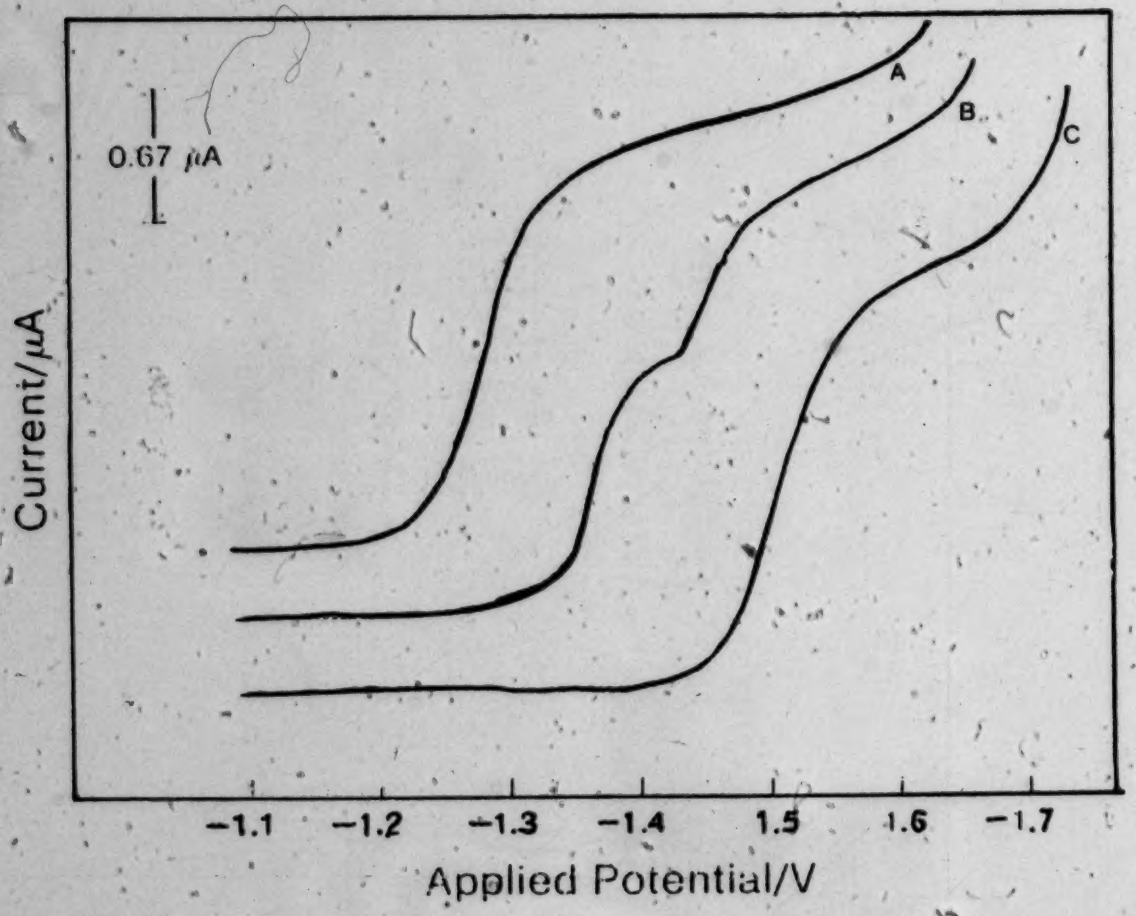
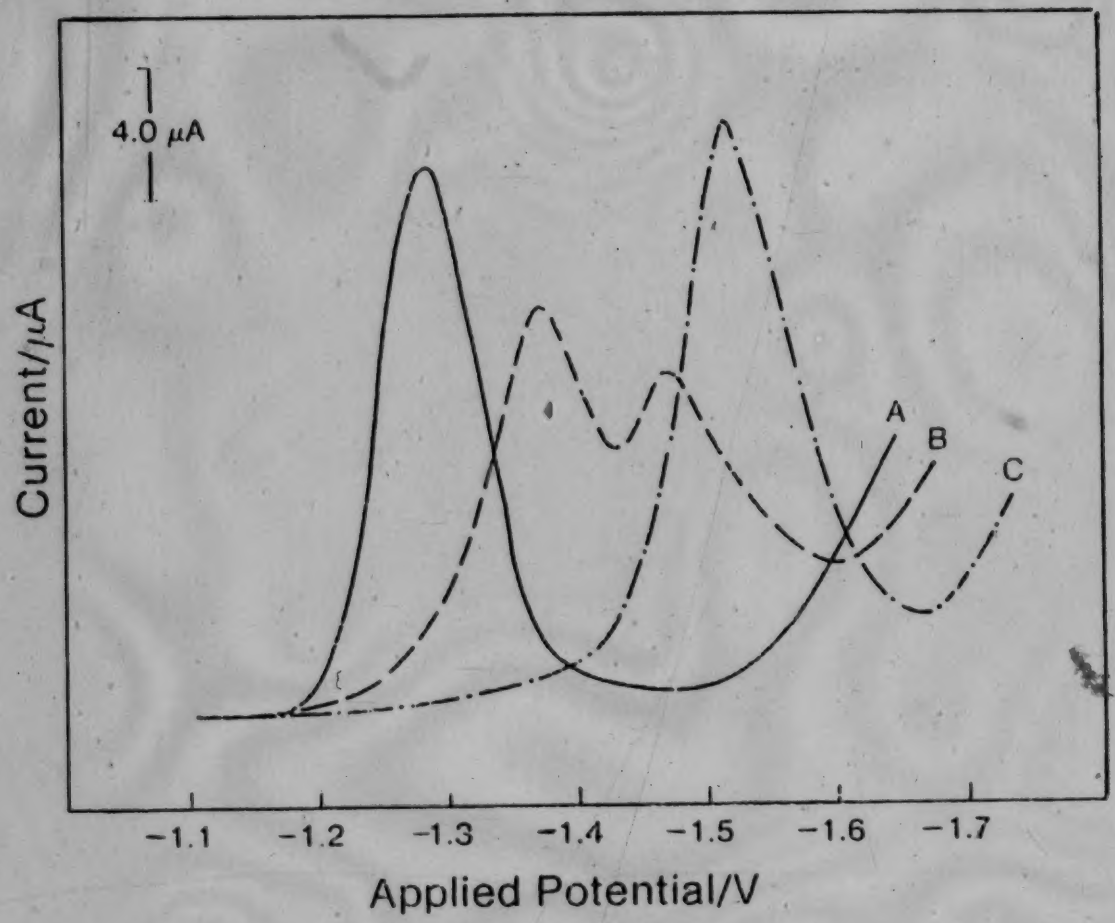


FIGURE 22: D.P. Polarographic Waves for Zomepirac Sodium in Britton- Robinson Buffer, pH Range A; 6.0, B; 8.0, C; 11.0.



in acidic media all the compounds investigated gave one diffusion controlled pH-dependent cathodic wave and a more negative pH-independent wave. The diffusion current constant increased with increasing pH to a maximum value at pH 9.0 and then decreased corresponding to a variation in the number of electrons transferred from one to two and then back to one. At a higher pH, a third more negative wave appeared. They found that the only isolatable products are pinacols (wave I in acidic media) and carbinols (waves II and III and the combined wave between pH 6.0 and 9.0). The electrochemical reduction of ketones is best explained by a free radical mechanism; in acidic and neutral media the ketone diffuses into the electrode field and its carbonyl group is polarized. Simultaneously, the carbonyl oxygen (now partially charged) attracts a proton, which process favours increased polarization. The protonated ketone completes its diffusion into the electrode interphase and acquires one electron resulting in wave I. The free radical produced can dimerize to the pinacol. A further reduction of the free radical to the carbinol result in wave II, which occurs at more negative potentials. Owing to the pH dependence of wave I, the two waves merge at higher pH's to yield a combined wave. In alkaline media the mechanism is essentially the same, except that because of the relative proton scarcity, carbinolate free radical ion is formed along with the carbinol free radical. The more difficult reduction of the former accounts for the decreased magnitude of the combined

wave and the appearance of wave III. The proposed mechanism is evaluated on the basis of the expected effects of structure. Generally, the electrochemical reduction of carbonyl groups is somewhat difficult to effect. The process has been found to be pH, concentration, and nature of the electrode dependent. The reduction product can be predicted if chosen conditions are applied (26).

Reduction of aldehydes and ketones to the corresponding alcohol is favoured by:

(1) Low concentration of starting material.

(2) Alkaline media, for example, potassium hydroxide and tetrabutylammonium hydroxide.

(3) A high overvoltage cathode such as mercury. While reduction to pinacols is promoted by:

(i) high concentration of starting meterial

(ii) acidic media

(iii) high current density

(iv) use of cathodes such as tin and copper.

Structural effects are generally excellent criteria for evaluating a mechanism with respect to its generality and /or veracity. Changes in molecular structure has a marked effect on carbonyl group reducibility. Substitutions of phenyl for methyl or hydrogen in acetaldehyde shifts the E to much less negative potentials. In acetophenone, replacement of methyl by _t_-butyl or isopropyl groups makes the $E_{1/2}$ more negative. The shifts of $E_{1/2}$ values in carbonyl group reduction can be explained by electronic effects and

to some extent by structural considerations. The role of the inductive effect, +I in ketonic reduction becomes readily apparent upon considering the decreasing ease of reduction in alkaline media for the three simple carbonyl compounds. Formaldehyde > acetaldehyde > acetone. This order is identical with the variation of the +I of the substituent groups. Analogously, benzaldehyde is reduced more readily than acetophenone since the phenyl group has a very strong +I (much stronger than H or methyl).

Evaluation of steric effects in correlating shifts of $E_{\frac{1}{2}}$ with structure presents some difficulty, since there is no definite way to measure these effects.

At the time of reduction, the carbonyl group is assumed to be perpendicular to a plane tangent to the electrode surface with the carbonyl oxygen furthest from the electrode, as expected from coulombic effects. Using a Fisher-Hirschfelder-Taylor model of the reacting molecules, the nearest possible approach to the electrode was determined by assuming several orientations for the substituents on the carbonyl carbon, placing the molecule on a smooth surface and measuring the distance from the surface (which represents the electrode) to the carbonyl carbon. The steric factors to consider include :

(1) nearest possible approach to the electode, assuming no distortion of bond angles by the electrode field.

(2) shielding of both the carbonyl carbon from back side attack by electrons and the carbonyl oxygen from front

side proton attack.

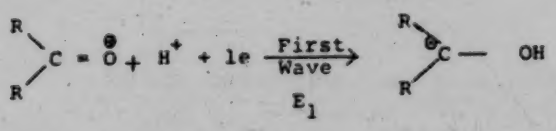
(3) relatively free rotation about the C-C bonds of the carbonyl group.

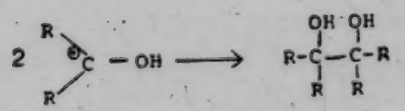
(4) hydrogen bonds.

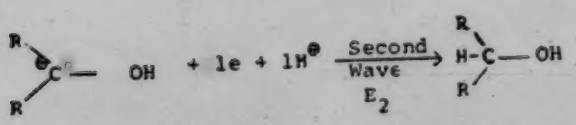
The facile reduction of benzophenone suggests that +I is the predominoant factor and that steric considerations are of minor importance.

In aqueous medium; the mechanism of the reduction of aldehydes and ketones has been postulated as follows(26):

Under acidic conditions two one-electron polarographic waves are observed

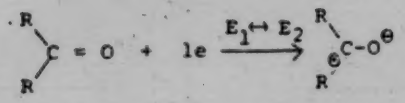


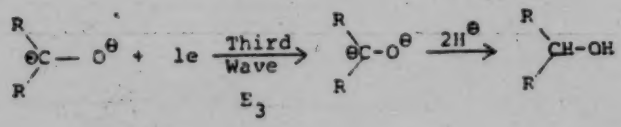




The dimerization, or further reduction of the ketyl radical depends on the applied potential. Thus, under macroscale conditions, with reduction at the plateau of the 1st wave, the main product is a pinacol, while the reduction at the plateau of the second wave affords the corresponding alcohol.

As the pH of the medium increases, the two waves merge and at a more negative potential a third wave appears.





The UV absorption spectra of zomepirac sodium in alkaline media shows two maxima. The first exhibited a λmax at 260nm and a $\log\varepsilon$= 3.94 while the λmax of the second was at 328nm, and the $\log\varepsilon$= 4.04. The reduced product shows only one maximum at 228nm with a $\log\varepsilon$= 3.97. (see Figure 23). Concentration of the reduced product was based on the initial concentration of zomepirac sodium taken.

The reduced product is unstable in acid and decomposes rapidly to give a reddish-pink precipitate. IR and NMR spectra were obtained on samples of the freeze dried product from the macroscale electrolysis. In acetone-d_6/D_2O, zomepirac sodium exhibits five peaks on the NMR(60MHz) with delta values at 1.6 (s 3H), 3.4 (s 2H) 3.55 (s 3H), 5.9 (s 1H) and 7.3 (quartet 4H). In the same solvent the reduced product exhibits six peaks on the NMR(200MHz) with delta values at 2.5 (s 3H), 3.8 (s 3H), 3.95 (d 2H), 6.3 (s 1H),

FIGURE 23: Ultraviolet Absorption Spectra of A, Zomepirac Sodium;
B, Electrolysed Zomepirac Sodium

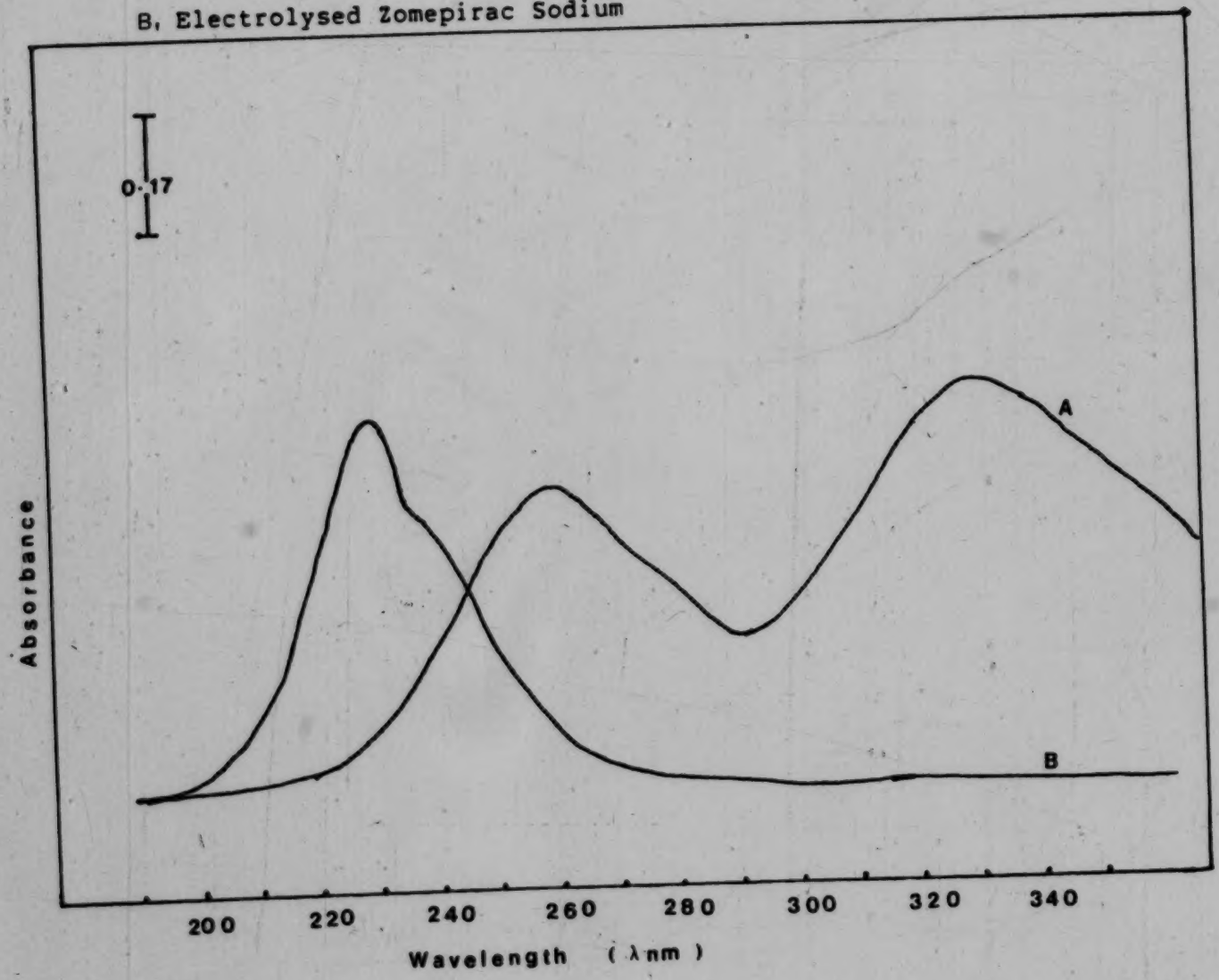


FIGURE 24: NMR Spectrum of Zomepirac Sodium in DMSO-d_6

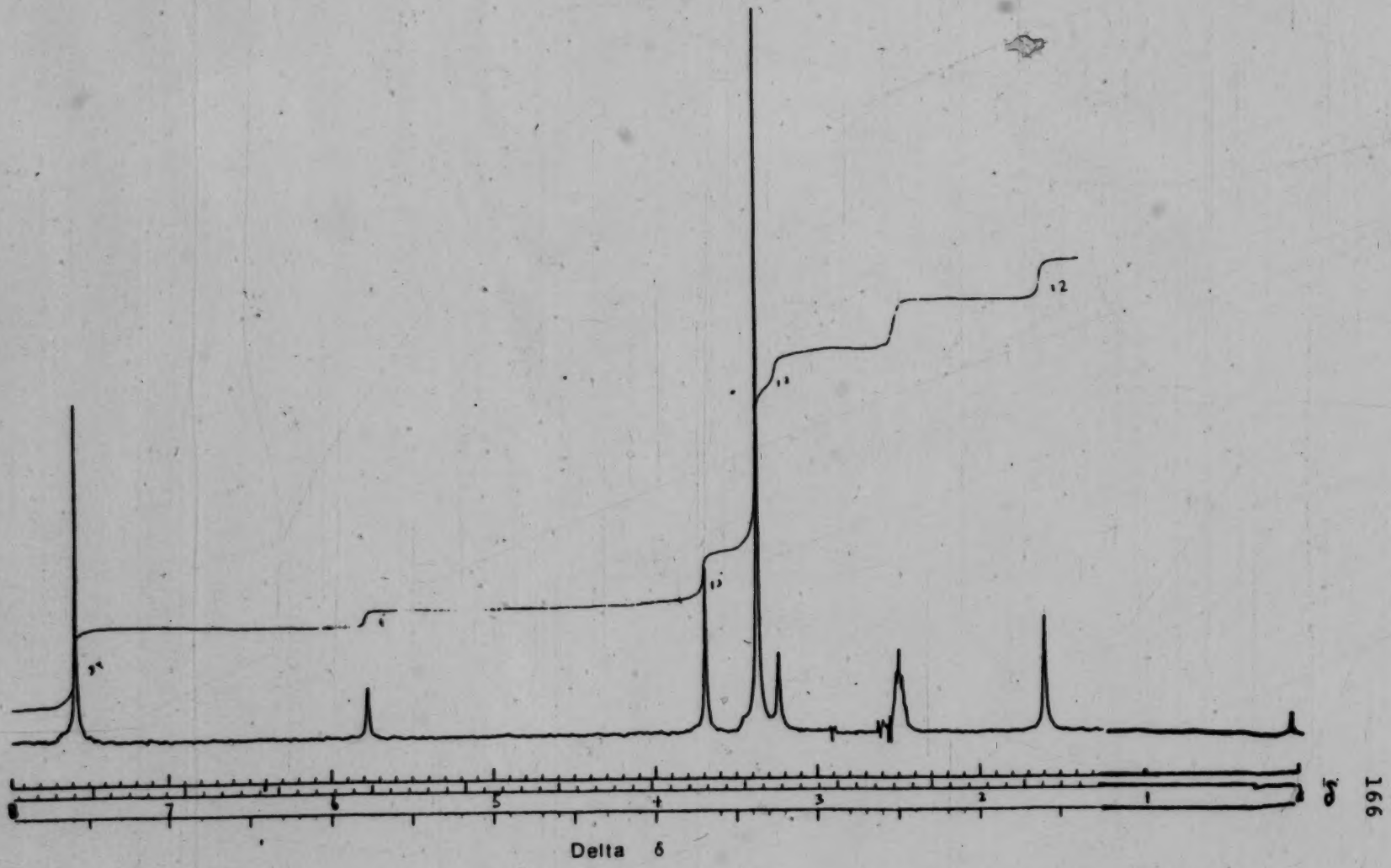


FIGURE 25: NMR Spectrum of Zomepirac Free Acid in DMSO-d_6

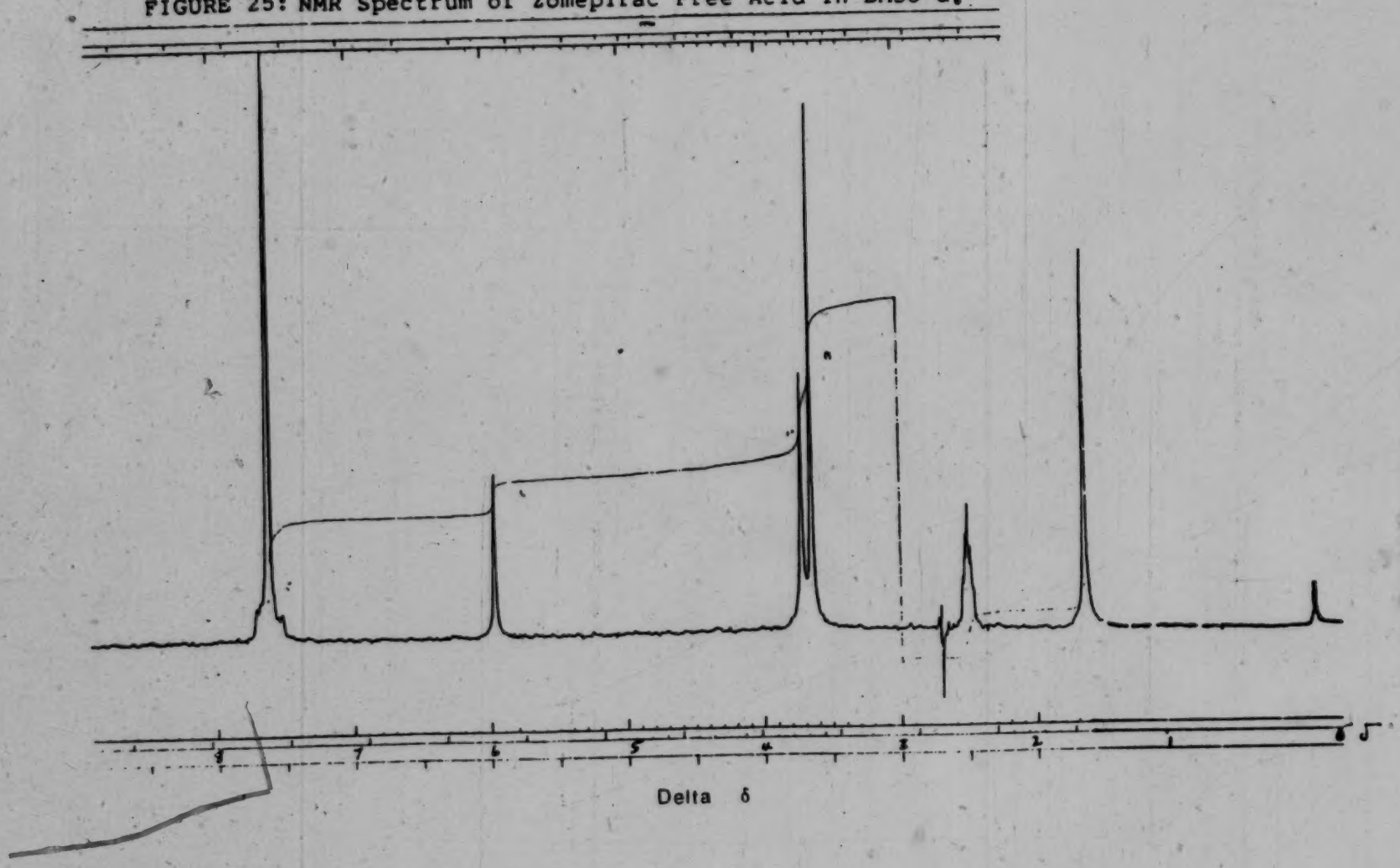


FIGURE 26: NMR Spectrum of reduced Zomepirac Sodium in Acetone-d_6-D_2O

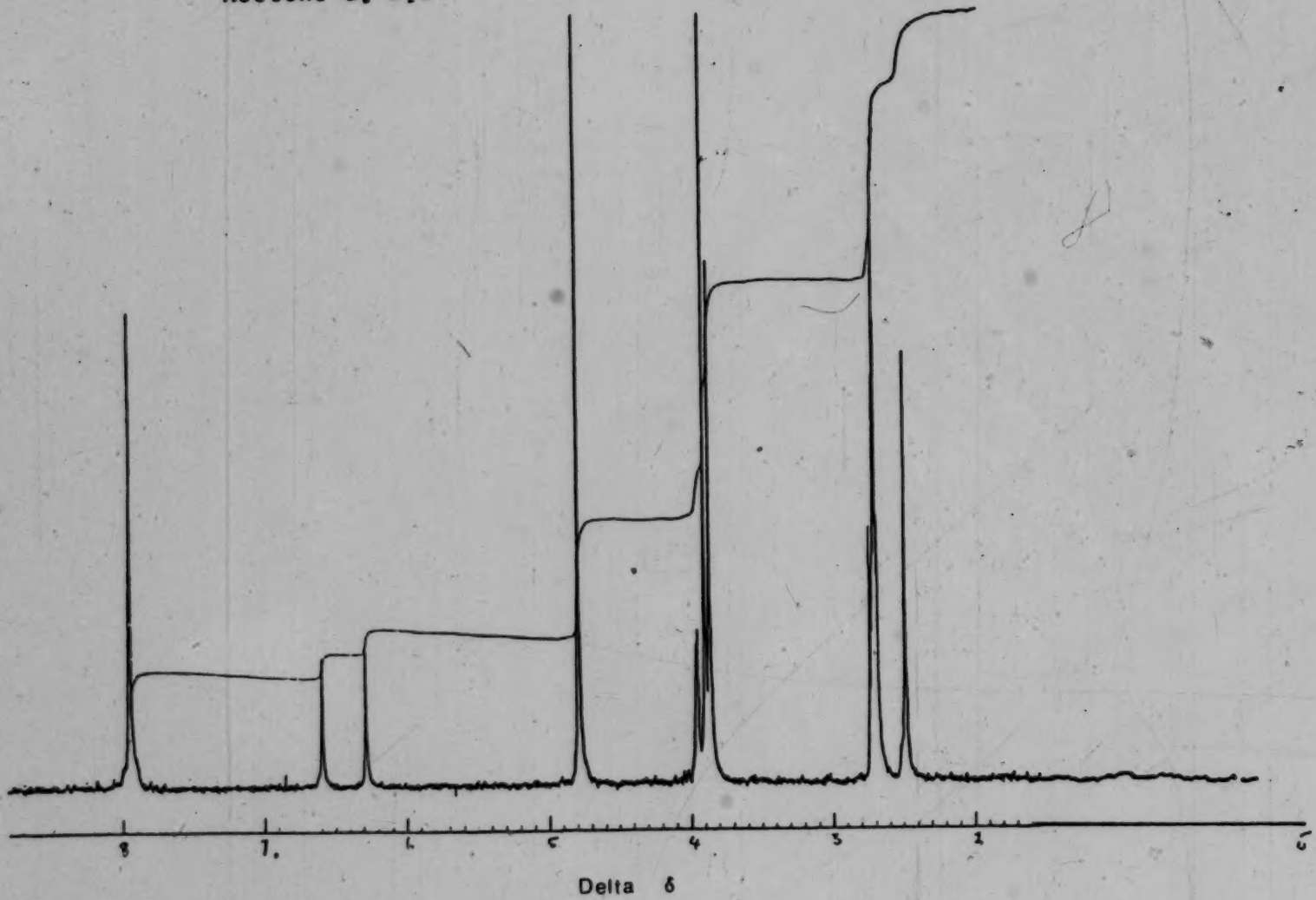
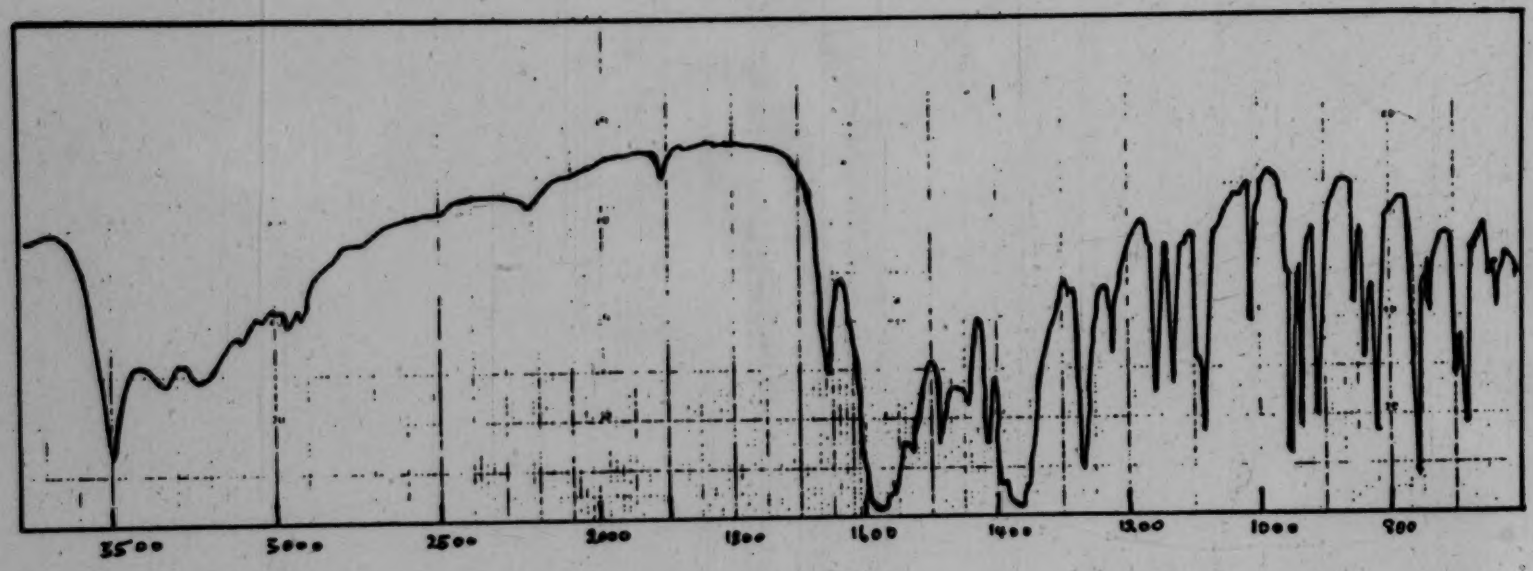


FIGURE 27: IR Spectrum of Zomepirac Sodium



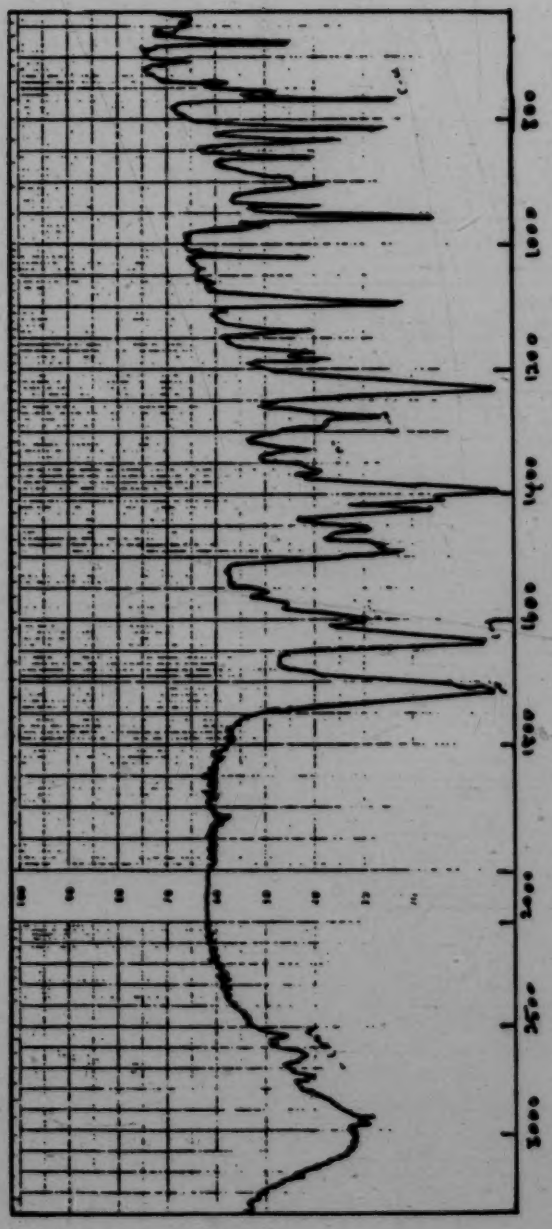


FIGURE 28: IR Spectrum of Zomepirac Free Acid

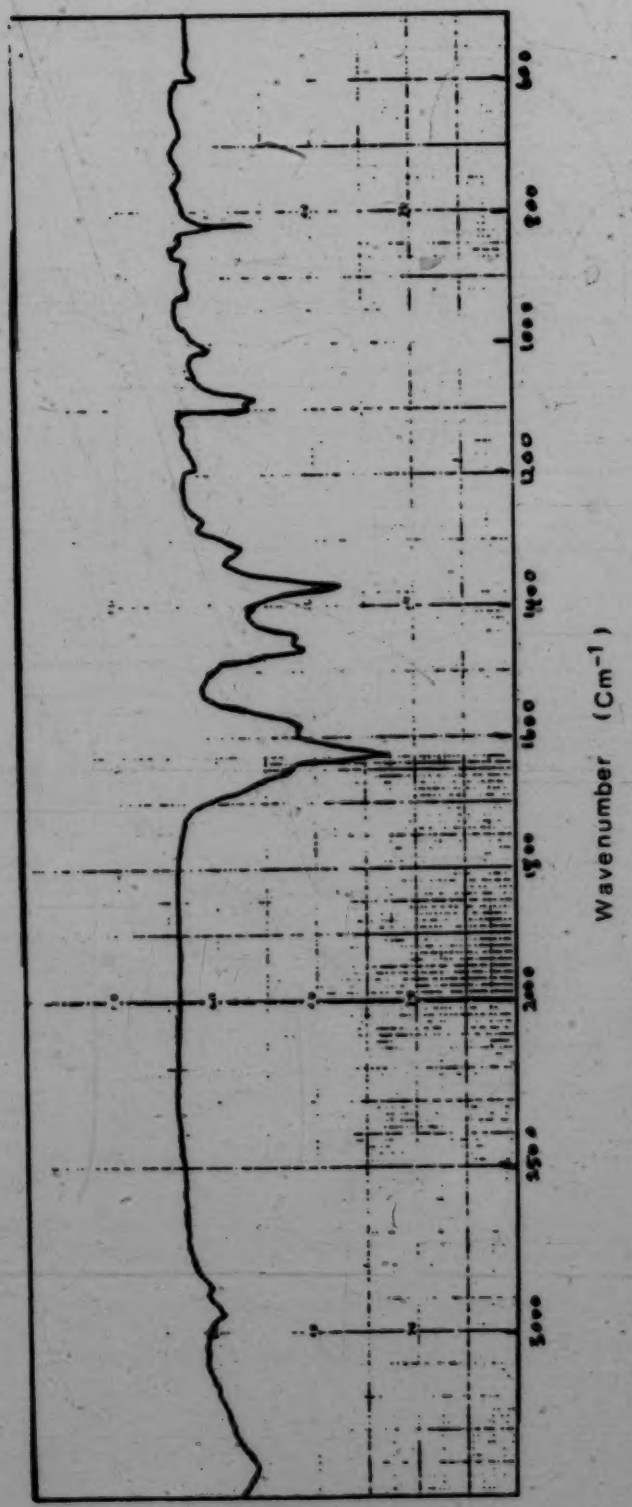


FIGURE 29: IR Spectrum of Reduced Zomepirac Sodium

6.6 (s 1H) and 7.95 (m 4H) (see Figures 24-29).

The IR (KBr) spectra (Figure 29) of the reduced product of zomepirac sodium shows an absence of the carbonyl peak at about 1600 1640/cm which is present in the IR spectra (Figure 27) of zomepirac sodium itself . The absence of the carbonyl group in the reduced product is also indicated by the disappearance of a UV absorption peak (Figure 23) in the range 250-350nm. The NMR peak at 6.6 owing to a single proton observed for the product confirms that the carbonyl group is the site of the electroreduction process (Figure 26). A reduction at the carbon-chlorine bond will also result to a product which has an additional proton, but the NMR peak for the added proton would have been further down field since it would have been an aromatic proton.

From the results obtained, we suggest the following pathway (Figure 30) for the reduction.

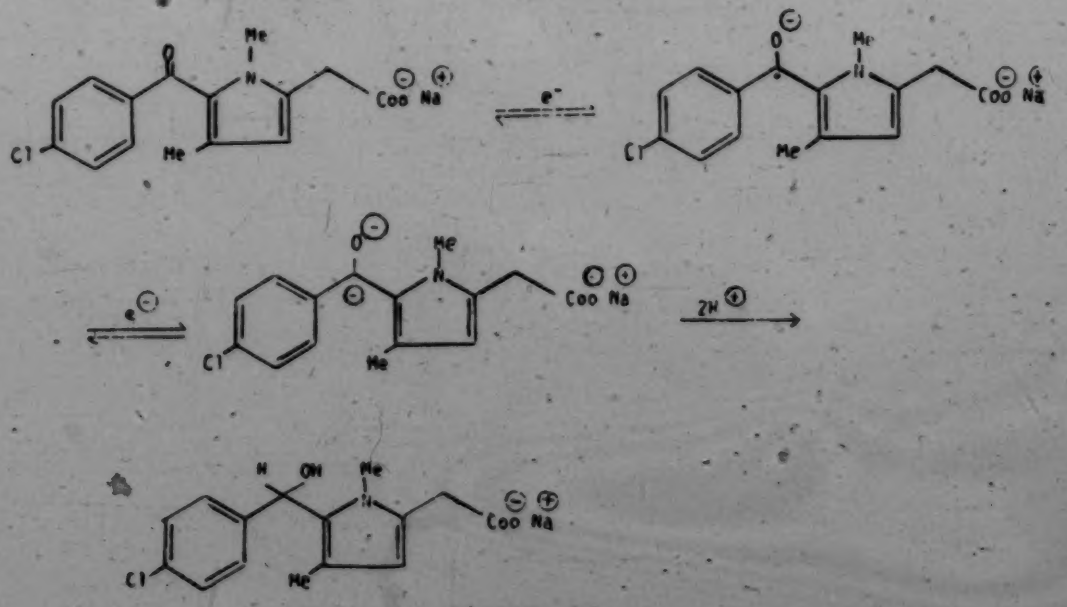


Figure 30

3.5.13 Polarographic Behaviour of Deschlorozomepirac

Deschlorozomepirac exhibits similar polarographic waves as those observed for zomepirac in Britton-Robinson buffer over the pH range 6.0 to 11.0. The deschlorozomepirac waves are affected by pH in the same manner as those for zomepirac. Consequently, the carbon- chlorine bond in zomepirac is not involved in the electrochemical reduction of the compound in aqueous systems.

3.5.14 Voltammetric Behaviour of Ketoprofen

Ketoprofen also exhibits three polarographic waves in Britton Robinson buffer over the pH range 5.0 to 11.0. At pH 6.0, a single well resolvd pH-dependent wave is observed with an $E_{1/2}$ value at -1.15V (see curve A, Figure 31). The $E_{1/2}$ shifts cathodically while the peak current decreases with increasing pH. The second wave is pH-independent and is observed within the pH range 6.5 to 9.0 (Figure 31, curve B). The $E_{1/2}$ value occurs at -1.39V. At pH 10.0, the two waves merge to form a well-resolved third wave which is also pH-dependent as evidenced by the $E_{1/2}$ value which shifts cathodically with increasing pH. At pH 11.0, its $E_{1/2}$ value is -1.44V (Figure 31, curve C). The electrochemical processes at the D.M.E. giving rise to these waves are considered to be identical to those for zomepirac sodium and, consequently, will not be repeated here. The graph of diffusion current versus the square root of corrected height of mercury column is a straight line but does not pass

through the origin (Figure 4). See Table 6 for the experimental data. The slope of this graph is 0.285μA/cm$^{1/2}$, while that for zomepirac sodium (Figure 3) is 0.228μA/cm$^{1/2}$. In Britton-Robinson buffer, pH 6.0, only one cyclic voltammetric reduction peak was observed exhibiting an E_p at -1.23V (Figure 32). A peak potential shift of 60mV was observed when the scan rate (ν) was varied from 200 to 10mV/s. The graph of $i_p/\nu^{1/2}$ versus ν is almost independent of scan rate, indicating a non-complicated slow electron transfer and homogeneous reaction which is also predominantly diffusion controlled (see Figure 33)(29). Table 7 exhibits the experimental data.

In dimethylformamide/tetraethylammonium bromide solution, ketoprofen exhibits two cathodic and one anodic cyclic voltammetric peaks. The E_{pc} values occur at -1.66V and -1.92V while the E_{pa} value is -1.76V (Figure 24). The height of all peaks decrease in the same proportion with decreasing scan rate. On continuous scanning at 200mV/s , however, the first cathodic peak height decreased more markedly than that of the second cathodic or the anodic peaks. The coulometric analysis indicated that two electrons per molecule were transferred in the electrochemical process. From the foregoing observations, two one electron transfer steps are predicted for which the first step is the rate determining step for the over all process.

The polarographic behaviour of ketoprofen at the D.M.E. has been found to be consistent with the reduction of a

Table : 6

Data for Diffusion Dependence Studies of Ketoprofen
Britton-Robinson Buffer, pH 6.0

Height Hg column (cm) h_0	m = mg of Hg/ sec	$m^{\frac{1}{3}}$	$t^{\frac{1}{3}}$	$h_b = 3.1/m^{\frac{1}{3}}t^{\frac{1}{3}}$	Corrected height h	$h^{\frac{1}{2}}$	i_p(μA)
80.0	1.32	1.09	1.26	2.24	77.76	8.81	2.30
75.0	1.29	1.08	1.26	2.26	72.74	8.52	2.25
70.0	1.27	1.08	1.26	2.27	67.73	8.22	2.25
65.0	1.11	1.03	1.26	2.37	62.63	7.91	2.10
60.0	0.97	0.98	1.26	2.48	57.52	7.58	1.98

Table : 7

Data for Determining the Effect of Scan Rate on the Cyclic Voltammetric Peak Current of Ketoprofen in Britton-Robinson Buffer, pH 6.0

Current i_p (μA)	Scan rate v mV/s	$v^{1/2}$	$i_p/v^{1/2}$
8.70	200	14.14	0.62
6.02	100	10.0	0.60
3.50	50	7.07	0.50
2.2	20	4.47	0.49

FIGURE 31: D.P. Polarographic Waves for Ketoprofen in Britton-Robinson Buffer, pH Range A; 6.0, B; 7.0, C; 11.0.

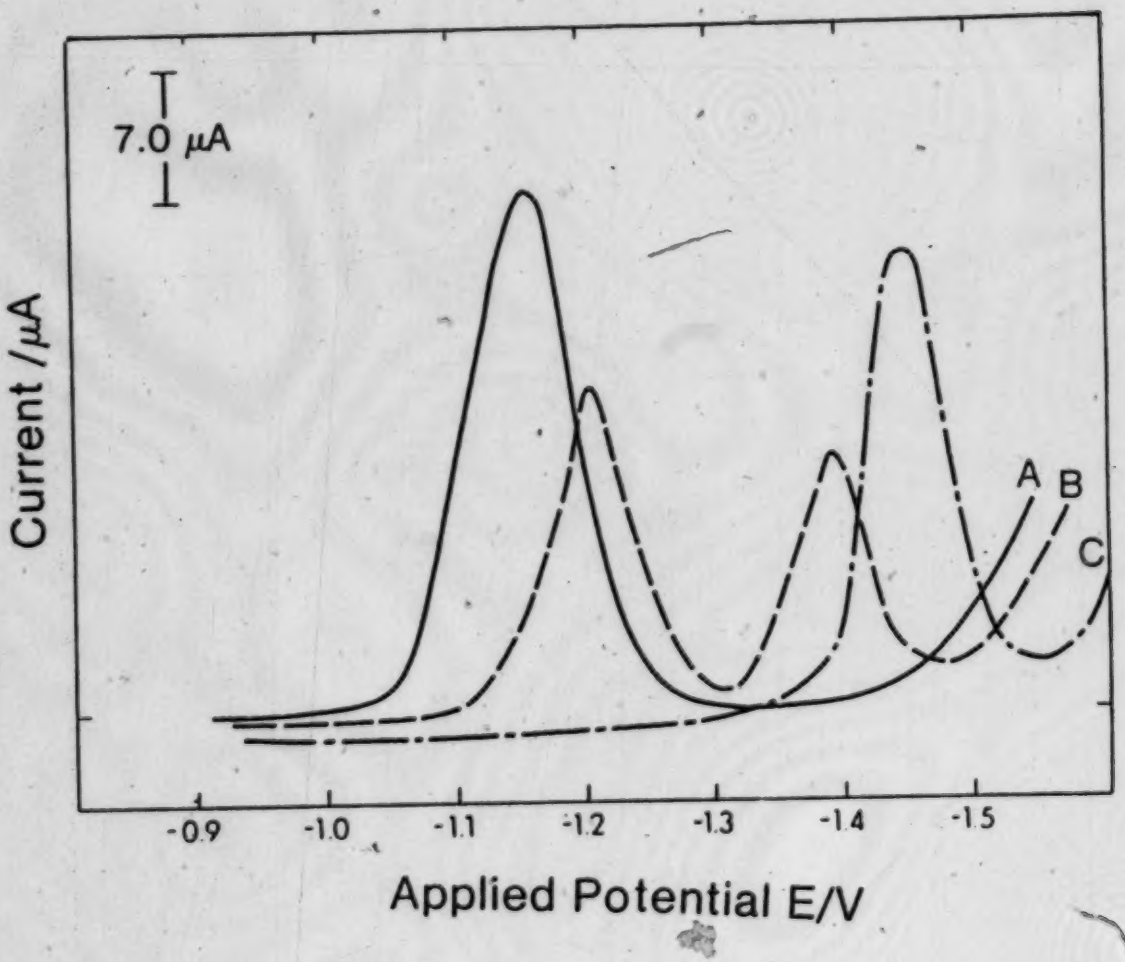


FIGURE 32: Cyclic Voltammogram of Ketoprofen in Britton-Robinson Buffer, pH 6.0

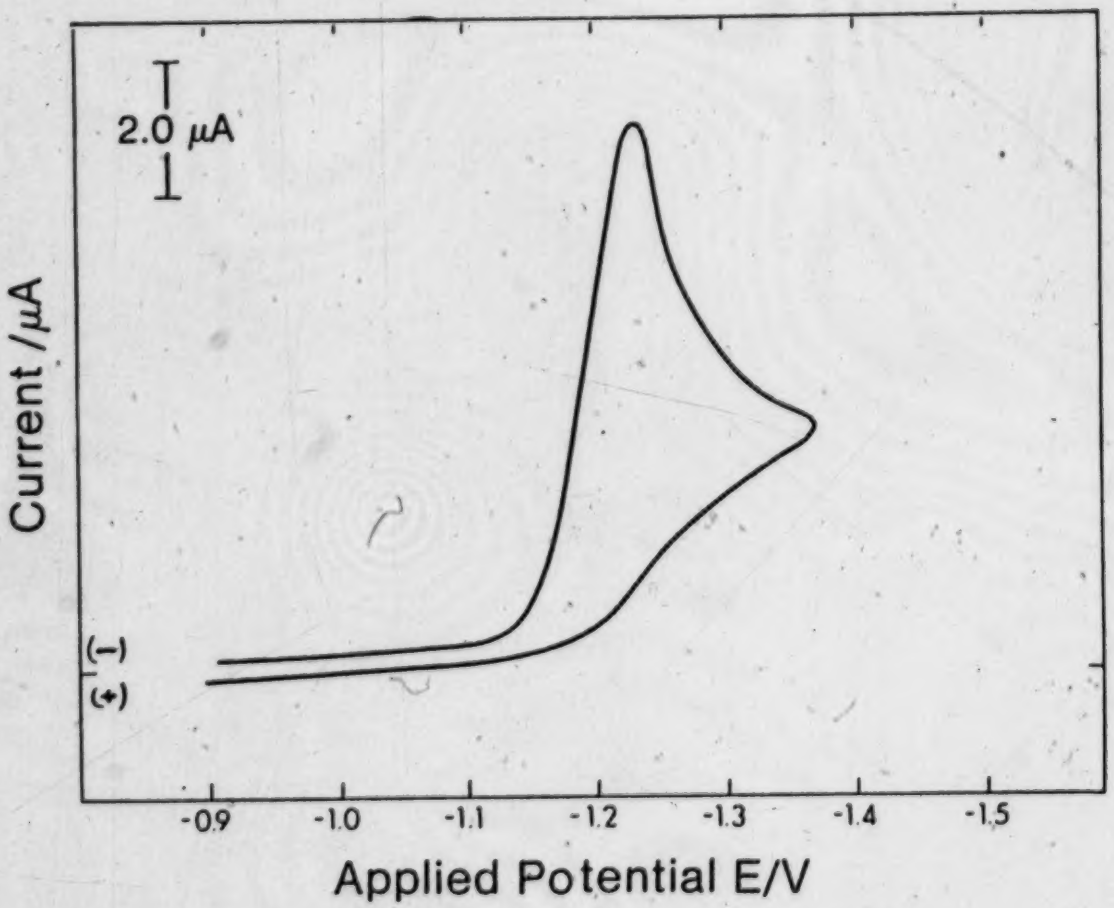
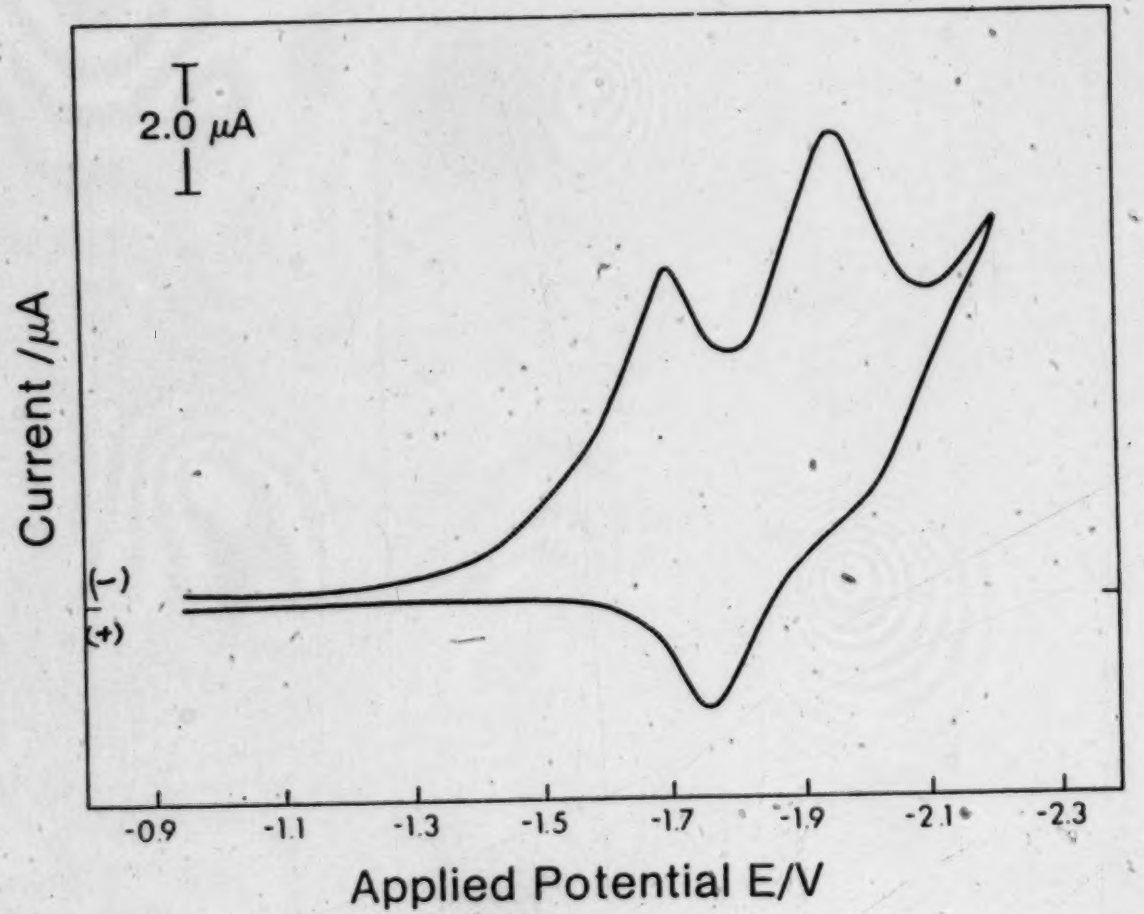


FIGURE 33: Graph of $i_p/v^{1/2}$ <u>versus</u> v for Ketoprofen in Britton-Robinson Buffer, pH 6.0

FIGURE 34: Cyclic Voltammogram of Ketoprofen in DMF/TEAB.



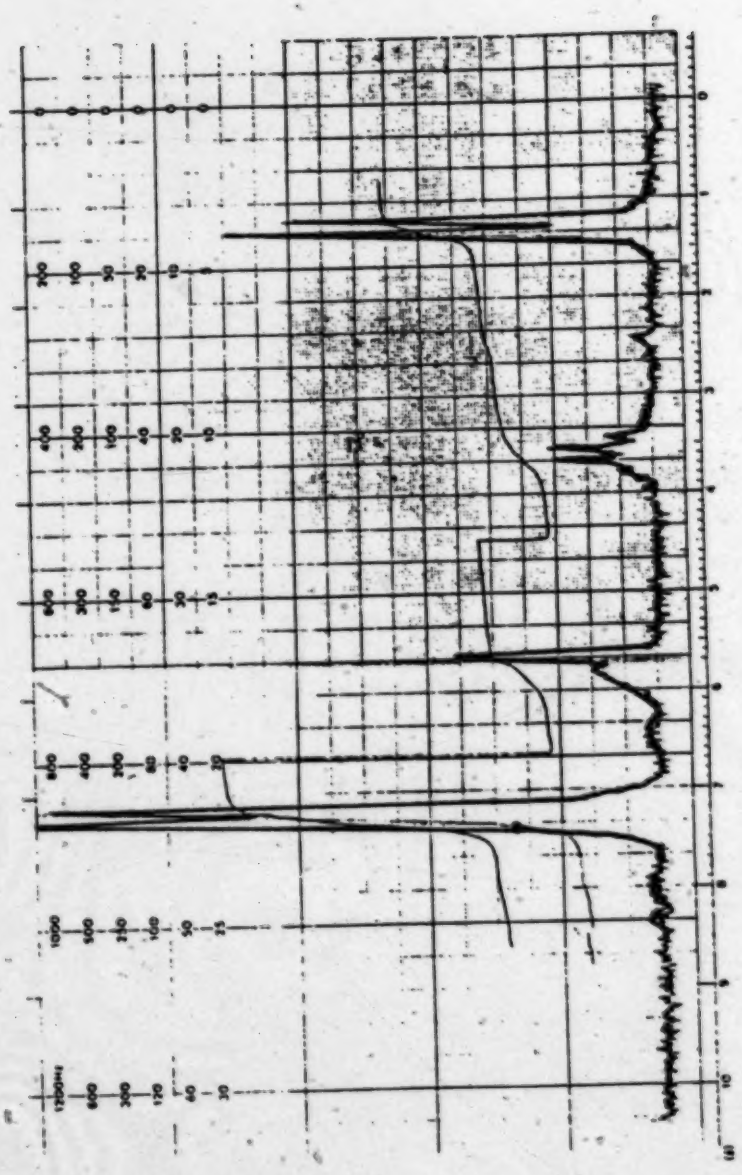


FIGURE 35: NMR Spectrum of Reduced Ketoprofen in DMSO-d_6

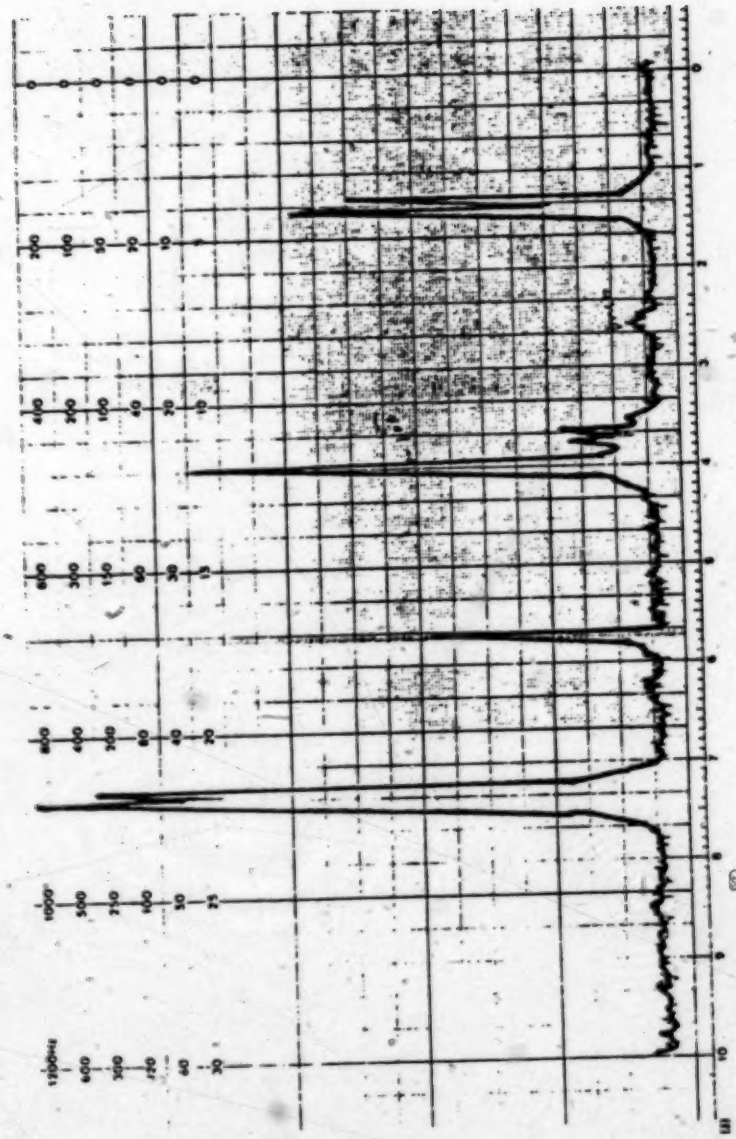


FIGURE 36: NMR Spectrum of Reduced Ketoprofen in DMSO-d_6/D_2O

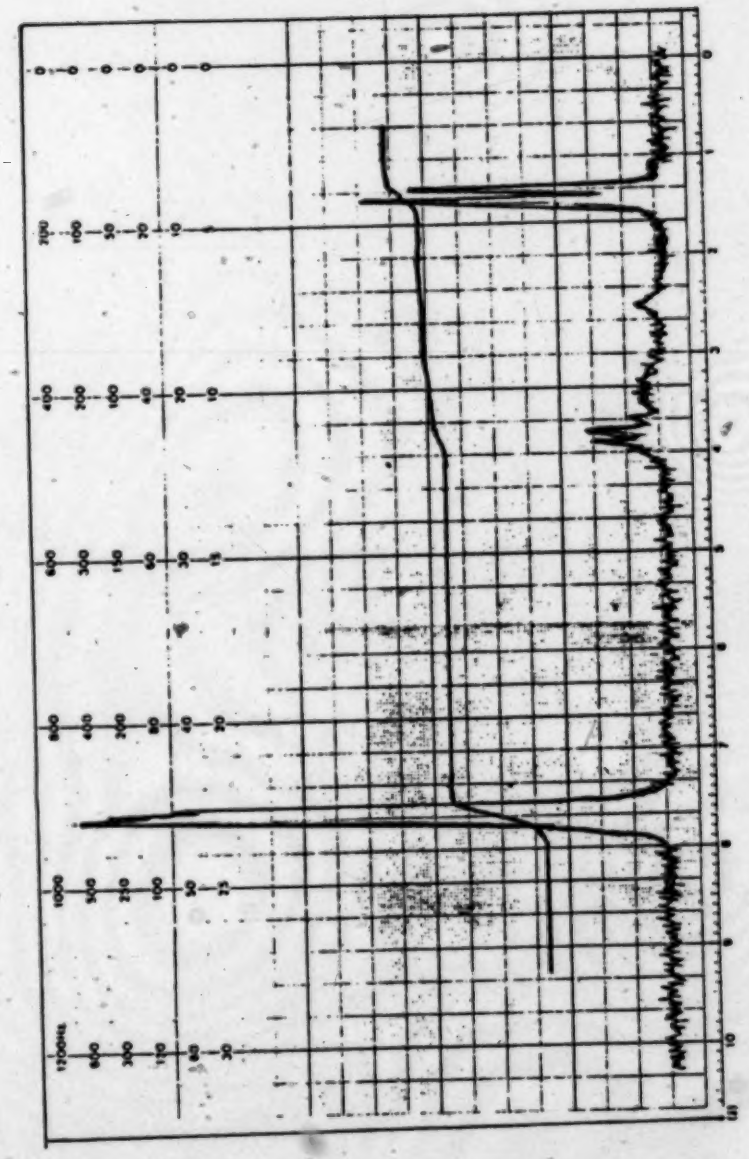


FIGURE 37: NMR Spectrum of Ketoprofen in DMSO-d$_6$

FIGURE 38: IR Spectrum of Ketoprofen

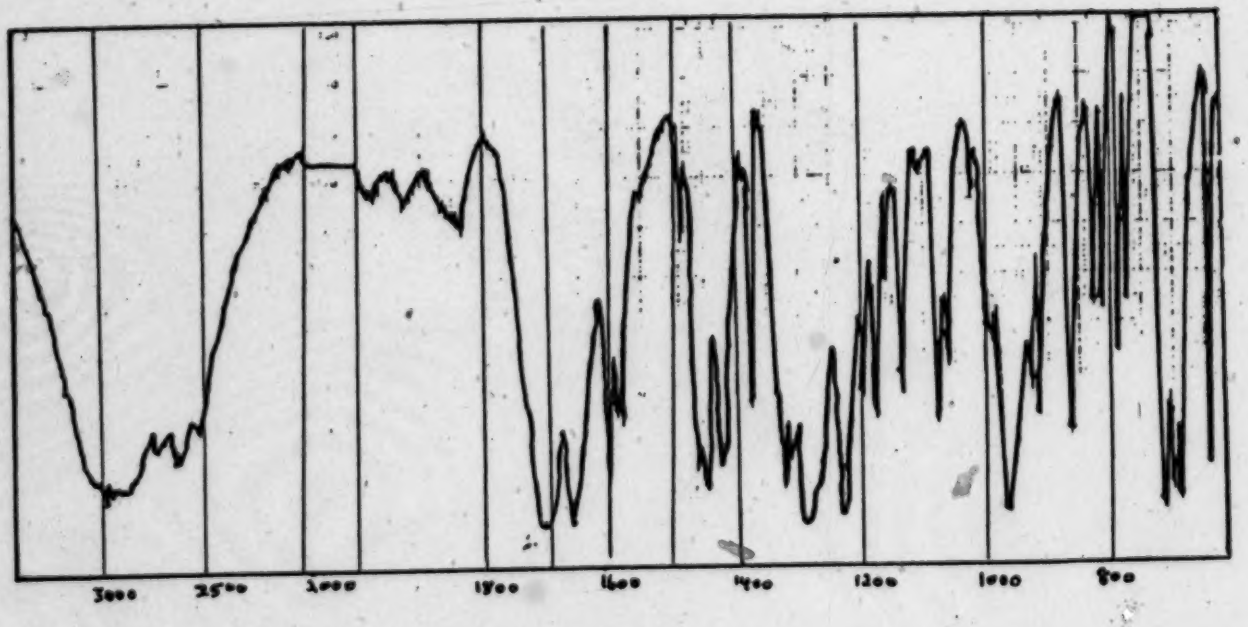


FIGURE 39: IR Spectrum of Reduced Ketoprofen

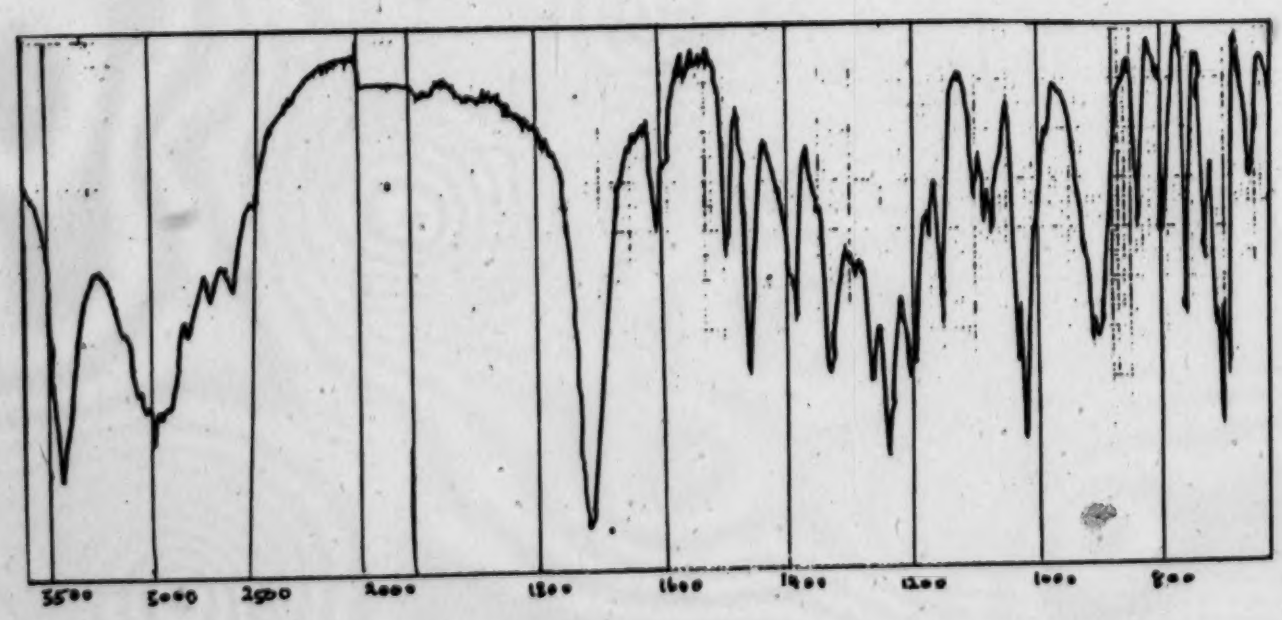


FIGURE 40: Mass Spectrum of Reduced Ketoprofen

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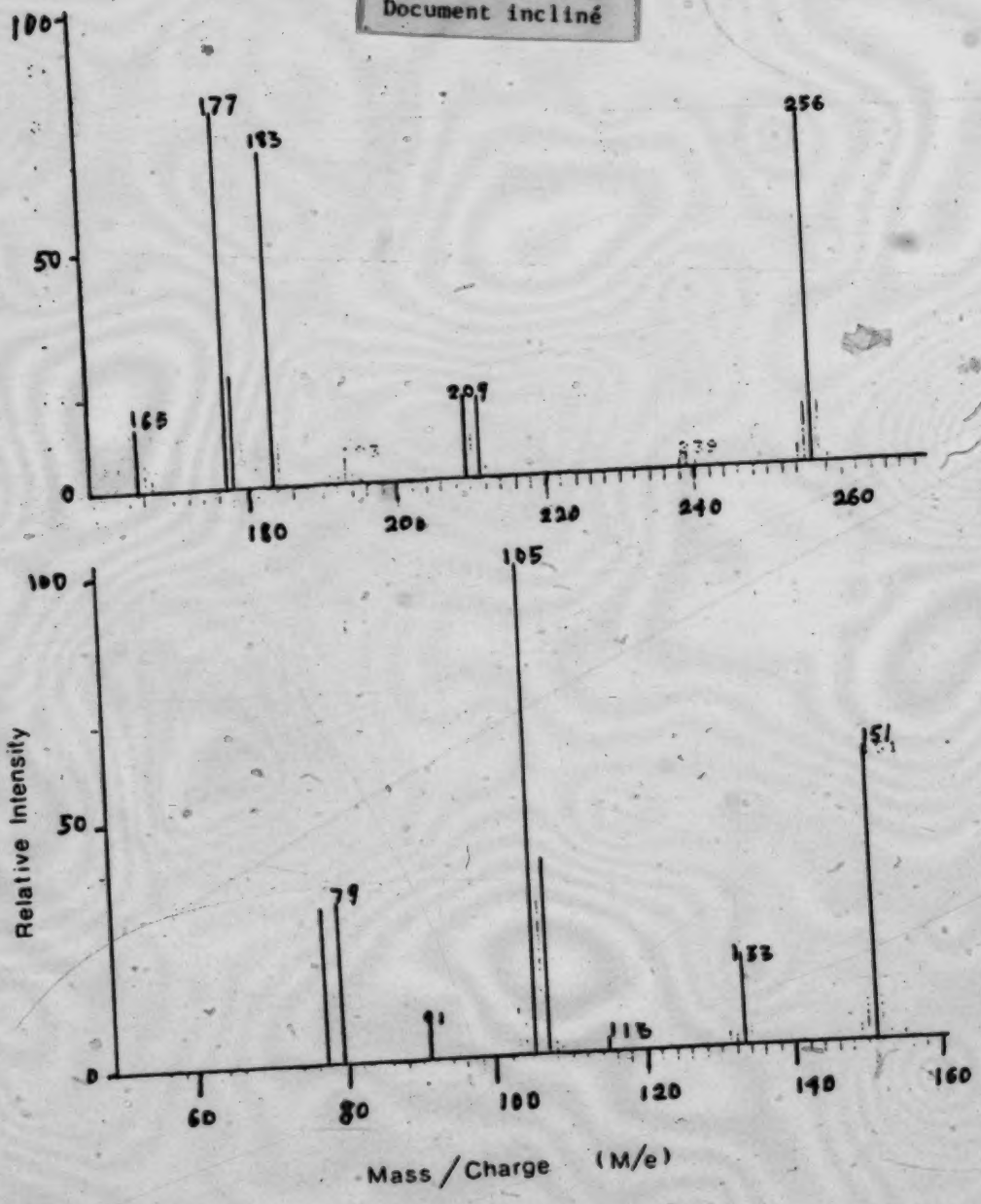
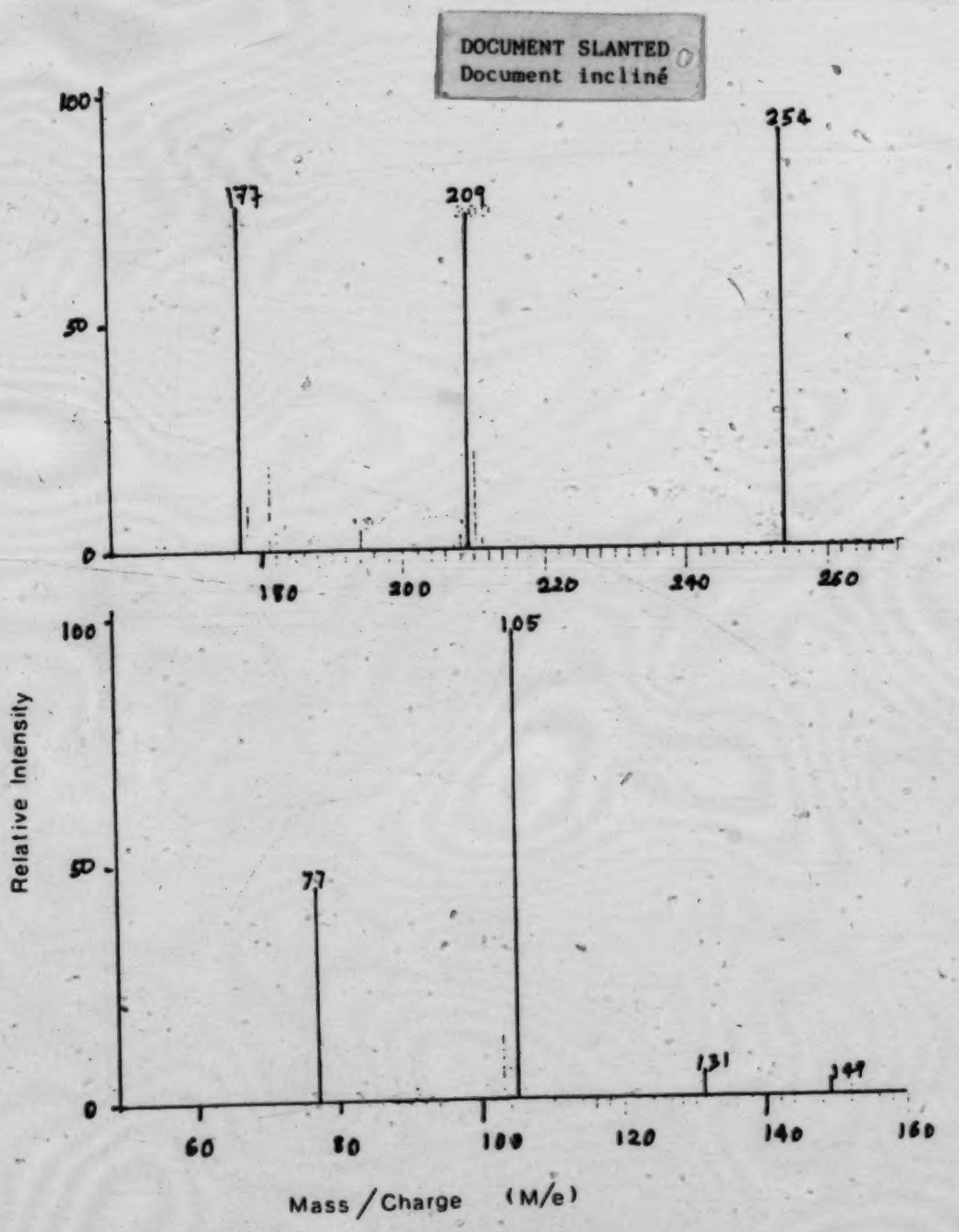


FIGURE 41: Mass Spectrum of Ketoprofen



carbonyl group. In DMSO-d_6-D_2O system, the NMR peak at 5.6δ is reduced to a singlet with an integration value corresponding to one proton (see Figures 35-36). Figure 37 illustrates the NMR spectrum of ketoprofen. The strong IR peak at 3420/cm is indicative of the presence of a hydroxyl group in the reduced species (Figures 38-39). The absence of the carbonyl peak at about 1670/cm ,(Figure 39) in the IR spectrum and the decrease in the UV absorbance peak at 252nm also indicates that the carbonyl group is the site of the electrochemical reduction. The molecular ion m/e ratio of 256 for the reduction product as compared with a value of 254 for ketoprofen (Figures 40 and 41 respectively) agrees excellently with the postulation that an alcohol is the electrochemical reduction product of ketroprofen.

From all the foregoing observations, the reduced product of ketoprofen is probably 2-(3-benzhydrolyl) propionic acid (Figure 42).

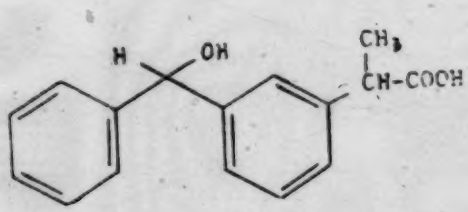


Figure 42

3.5.15 Analysis of Pharmaceutical Dosage Forms of Zomepirac Sodium

Table 8 gives the results of the assay of zomepirac sodium tablets based on the average value obtained with a

sample of twenty tablets. The standard deviations of the method together with the analysis obtained by the manufacturer's quality control laboratory are presented for comparative purposes. The assay provides results that are equally comparable to those of the manufacturer, at both pH values of 11.0 and 6.0.

Although the d.c wave could have been used for analytical purposes, the differential pulse wave is much better resolved, as illustrated in Figures 21 and 22. The proposed method has the advantage of simplicity, high sensitivity and rapidity. It has the same disadvantage as the official spectrophotometric method, however, in that certain degradation products, if present, could not be detected.

In Table 9, the average of the results obtained with ten individual tablets is presented.

An important step in working out an assay for drug content is an examination of the effects of other constituents present in the pharmaceutical preparations. Polarographic screening experiments showed that none of the excipients present in zomepirac sodium tablets cause any change in the shape or height of the polarographic wave of zomepirac. Thus, polarographic analysis can be performed without interference by simply pulverizing the tablet and dissolving the active ingredient in an appropriate medium.

Table : 8

Analysis of Zomepirac Sodium Tablets at two pH values

Labelled Claim 100 mg	Differential - Pulse Method pH 6.0	pH 11.0	Manufacturers UV Method
Amount found (%)	96.6 ± 0.2	98.7 ± 0.4	97.54

Table : 9

Average value for the Analysis of Single Zomepirac Tablets at pH 11.0

Labelled Claim 100 mg	Differential-Pulse Polarographic Method pH 11.0	Manufacturer UV Method
Average mg/tab found	100.7 ± 1.0	99.15

Table : 10

Data for Assay of Ketoprofen Capsules by D.P.P. in Britton -Robinson Buffer. pH 6.0

Sample No	D.P.P. Current(μA) 1	2	Average peak current (μA)	Ketoprofen Ref. Standard Current (μA)	% Recovery	mg/ capsule found	Recovery capsule found
1.0	9.7	9.8	9.75		93.75	46.87	
2.0	10.2	10.2	10.20		98.07	49.03	
3.0	10.3	10.3	10.30	10.40	99.04	49.52	49.73
4.0	10.9	11.2	11.0		105.76	52.88	
5.0	10.2	10.8	10.5		100.96	50.48	

Table : 11

Data for Content Uniformity Test for Ketoprofen Capsules by D.P.P in Britton-Robinson Butter, pH 6.0

Capsule No.	d.p.p peak current (μA) 1	2	Average peak current (μA)	Ketoprofen Ref Standard solution Peak current(μA)	% Recovery	mg/capsule found	Average mg/capsule found
1.0	11.6	11.4	11.50		100.87	50.43	
2.0	11.5	11.5	11.50		100.87	50.43	
3.0	11.7	11.6	11.65	11.4	102.19	51.09	
4.0	11.6	11.5	11.55		101.31	50.65	
5.0	11.0	11.3	11.15		97.80	48.90	49.9 ± 1.3
6.0	11.7	12.0	11.95		88.51*	44.25	
7.0	12.4	12.3	12.35		91.48*	45.74	
8.0	12.7	13.0	12.85		95.18	47.59	
9.0	13.2	13.0	12.85	13.5	97.03	48.51	
10.0	13.9	13.8	13.85		102.59	51.29	

± Standard deviation = 1.34

* neglected

Table : 12

Data for Analysis of Ketoprofen Suppositories (100 mg) in B.R.Buffer, pH 6.0

Suppository No.	d.p.p peak current(μA) 1	2	Average peak current (μA)	Amount found mg	Average Recovery mg
1	11.9	12.0	11.95	94.84	
2	12.5	12.5	12.50	99.20	
3	13.0	13.4	13.20	104.76	
4	12.9	12.8	12.85	101.84	
5	12.7	12.8	12.75	101.19	
6	12.8	12.7	12.75	101.19	101.53 ± 3.6
7	12.3	12.3	12.30	97.61	
9	13.4	13.5	13.45	106.74	
10	13.3	13.2	13.25	105.15	

Table : 13

Assay of Ketoprofen Capsules by Differential-Pulse Polarography

In Britton-Robinson Buffer, (pH 6.0) - Methanolic System

Capsule Lot No	Label Claim(mg)	Recovery by Manufacturer (mg)	% Recovery by Manufacturer	Recovery by d.p.p (mg*)	% Recovery by d.p.p
247	50	50.07	100.1	49.7	99.4

* Each value is the average of five determinations

Table : 14

Average Value for the Analysis of Ten Individual Ketoprofen Capsules and Suppositiories in Britton-Robinson Buffer, (pH 6.00)- Methanolic System

Dosage Form	Label claim/mg	% Recovery by Manufacturer/mg	% Recovery by d.p.p
Capsule. Lot 247	50	97.6	99.7 ± 1.3
Suppository. Lot 169	100	99.8	101.5 ± 3.6

3.5.16 Analysis of Pharmaceutical Dosage Forms of Ketoprofen

Both the d.p.p peaks resulting from waves A and C (Figure 31), are well-resolved and may be utilized for analysis of dosage forms, however, the d.p.p peak of wave A is highly reproducible and therefore was chosen for the analysis of ketoprofen dosage forms. The peak current varied linearly with the concentration of the drug over the range 1 x 10^{-4} to 5 x 10^{-4}M. Tables 10,11 and 12 illustrate the actual d.p.p. data for analysis of ketoprofen capsules and suppositories while Tables 13 and 14 provide the assay results for the two dosage forms investigated. Values obtained by the manufacturers quality control laboratory are also presented for comparison purposes, and an excellent agreement is observed between the two results. Common tablet excipients and suppository bases do not interfere with the method which is simple, sensitive and rapid. The method, or very simple variants of it, shows promise of general application to a series of similar aryl-alkanoic acid compounds associated with anti-inflammatory therapy.

3.6 Summary and Conclusions

(1) Simple differential-pulse polarographic methods have been developed for the detection and determination of two aryl-alkanoic acid antiinflammatory agents (zomepirac sodium and ketoprofen) and their pharmaceutical dosage forms. The methods utilized the electroreducibility of a carbonyl group present in the structure of these compounds. Excellent results were obtained when the methods were applied to the analysis of tablets and / or suppositories.

(2) The actual mechanism of the electroreduction has been postulated. Reduction products of zomepirac sodium and ketoprofen were isolated and characterised by UV, IR and NMR spectroscopic techniques.

(3) Studies of the effects of the composition of supporting electrolyte, the effect of pH on the system and of heights of the mercury column, have been conducted. The electroreduction processes have been found to be predominantly diffusion dependent in the ranges of mercury column heights and concentrations selected. A mercury column height of 75cm was used in each instance while Britton-Robinson buffers at pH 6.0 and 11.0 were used as supporting electrolytes for ketoprofen and zomepirac sodium respectively. All measurements were made by employing a saturated calomel electrode (SCE) as reference electrode and a platimum wire as the auxiliary electrode.The working electrode was a D.M.E. The $E_{\frac{1}{2}}$ value for the analytical wave for ketoprofen was -1.15V, while that for zomepirac sodium

was -1.5V.

(4) Linear and reproducible calibration graphs were obtained using pure reference standards of ketoprofen and zomepirac sodium supplied by the respective manufacturers. The differential-pulse polarographic determinations were based on linear relationships between diffusion currents and concentrations of each species. Blank determinations were required and the amounts of ketoprofen and zomepirac sodium in the respective dosage forms were calculated by the direct comparison method. Commonly used tablet excipients and supository bases were found not to interfere with the determinations.

(5) It is concluded that the accuracies, sensitivities, and reproducibilities of these d.p polarographic methods make them suitable for routine analysis of these drugs in dosage forms. The methods, or very simple variants, show promise of general applications to a series of similar aryl-alkanoic acid compounds associated with anti-inflammatory therapy.

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